Modification of tau pathology by yeast prion seeding and tau oligomer expression

Inauguraldissertation

zur

Erlangung der Würde eines Doktors der Philosophie

vorgelegt der

Philosophisch-Naturwissenschaftlichen Fakultät

der Universität Basel

von

Martin Flach

Basel, 2020

Genehmigt von der Philosophisch-Naturwissenschaftlichen Fakultät

auf Antrag von

PD Dr. Dr. David T. Winkler

Prof. Dr. Christoph Handschin

PD Dr. Paolo Paganetti

Basel, den 13.10.2020

_____

Prof. Dr. Martin Spiess, Dekan

Preface

The following dissertation was written by the author.

The “Results” section consists of two manuscripts.

In the first-authorship manuscript (Cross-seeding of Alzheimer-related prion-like proteins by non-mammalian prions) the author significantly contributed to experiments, analysis, and writing process. In the first co-authorship manuscript (Severe oligomeric tau toxicity can be reversed without long-term sequelae) the author significantly contributed to experiments and analysis.

The additional data section “Supplementary Results” is the result of own work.

Abstract

Neurodegenerative disorders are a mayor health risk for the aging population of the world. The aggregation of tau constitutes a mayor hallmark of Alzheimer disease (AD) and other neurological diseases such as progressive supranuclear palsy, corticobasal degeneration, and frontotemporal dementias with parkinsonism liked to chromosome 17. Tau spreads in a prion like manner via the formation of disease specific fibrils. The cause of tau aggregation in neurodegenerative disorders has yet remained unclear. We were now able to show, that the archaic yeast prion domain Sup35NM is able to cross-species cross-seed human tau. In tau transgenic P301S mice this led to an accentuation of tau pathology in the hippocampus of Sup35NM inoculated mice. *In vitro* seeding of tau monomers with Sup35NM fibrils led to the accelerated formation of tau fibrils. These fibrils showed a characteristic corkscrew-like pattern. In AD, early tau aggregation occurs in the olfactory bulb. The nasal sensors are in close contact to exogenous agents and the microbiome present in the nasopharyngeal cavity, and they are anatomically connected to the limbic system, which is also affected early in AD. Together our results allow a novel perception of the prion world, in which archaic prions of the human microbiome are able to influence the aggregation of prion-like proteins like tau in neurodegenerative diseases. Understanding the initiation of prion formation in sporadic neurodegenerative disorders will be pivotal for the long-awaited generation of preventive or curative approaches for these devastating disorders.

Another important aspect concerning the desired treatment of neurodegenerative diseases like tauopathies is the potential long term effect of neurotoxic stress caused by misfolded proteins like tau. We therefore investigated the long term effects of severe early neurotoxic tau stress in recovered transgenic P301SxTau62 mice. Counterintuitively, P301SxTAU62 tau mice which underwent transient severe paralysis in early life, and maintained their P301S tau expression, developed less tau pathology and even maintained a slightly better motor performance when aging, in comparison to their heterozygous P301S transgenic littermates, which were not exposed to early tau stress. In accordance with that, different from tau fibrils the tau oligomers detected in the brains of P301SxTAU62 tau mice were not able to cause tau fibrillization in human tau transgenic ALZ17 mice. This is further evidence, that the structure of tau aggregates plays a crucial role when it comes to tau pathology spreading in the brain via prion-like template transmission. It also speaks for the development of therapies which target tau before the formation of tau fibrils was initiated.

Table of contents

1 Introduction

1.1 Alzheimer disease, the amyloid cascade hypothesis and tauopathies

1.1.1 Alzheimer disease

Alzheimer Disease (AD) is the most common form of senile dementia (Gupta *et al.*, 2010) with age as the main risk factor. The incidence rate for AD increases from 0.08% per year in the age group of 60-65 to 6.48% incidence rate per year in the age group of 85+ (Kawas *et al.*, 2000). In 2013 in the US 84,767 deaths from AD were recorded, making it the sixth leading cause of death for the total population and the fifth leading cause of death for people older than 65 years (Gaugler *et al.*, 2016). Between 2000 and 2013 deaths from AD increased by 71% and with an aging population this rate is predicted to rise even further (Gaugler *et al.*, 2016). AD is characterized by two hallmark lesions: Extracellular plaques of amyloid-beta (Aβ) and the formation of neurofibrillary tangles (NFTs) and neuropil threads (NTs) by tau protein (Gaugler *et al.*, 2016). The Aβ plaques are caused by an alteration of amyloid precursor protein (APP). Aβ builds deposits in the cortex, the limbic system and the walls of arteries and arterioles where they cause cerebral amyloid angiopathy (CAA). Structural alterations of the microtubule associated protein tau (MAPT) lead to a loss of function and fibrillization and cause a significant loss of synapses and neuronal loss. (López and DeKosky, 2008; Ellison *et al.*, 2012). The clinical symptoms of AD start with deficits in recent memory and language difficulties, and will gradually progress to a global cognitive impairment. During the early and middle phases of AD development, the alertness of patients is well preserved, and motoric and sensory functions are mainly intact. However, as subjects continue to worsen cognitively, a slowing of motor functions like gait and coordination can often lead to motoric limitations comparable to the ones observed in Parkinson patients (López and DeKosky, 2008; Gupta *et al.*, 2010). Although over 90% of patients with AD appear to suffer from sporadic AD (SAD) (Chai, 2007) there are some genetic risk factors. Carriers of the apolipoprotein E, type 4 allele (APOE-ε4) have an increased risk to develop AD and a lower age of onset (Saunders *et al.*, 1993). People with Down syndrome have a higher risk to develop AD early in life. This abnormality has been correlated to the triplication and overexpression of the gene coding for APP, located on chromosome 21(Ricciarelli and Fedele, 2017).

Autosomal dominant AD has a prevalence of less than 1% and is caused by mutations in APP, from which Aβ is cleaved, or by mutations in presenilin 1 (PS1) and presenilin 2 (PS2) which are part of the γ-secretase complex cleaving APP (Van Cauwenberghe, Van Broeckhoven and Sleegers, 2016).

Besides the very low percentage of cases of autosomal dominant AD, the findings about APP, PS1 and PS2 were used for the formation of the amyloid cascade hypothesis, which is the subject of the next section.

1.1.2 The amyloid cascade hypothesis

The mutations in APP and PS1/2 in cases of familial AD (FAD), and the higher risk of early onset of AD for Down syndrome patients led to the development of the amyloid cascade hypothesis (Ricciarelli and Fedele, 2017). As FAD patients will develop the same pathological changes as SAD patients, including tau aggregations, it was thought that Aβ aggregation is the initiator of AD (Musiek and Holtzman, 2015). However, later it was shown that accumulation and deposition of Aβ are not correlating with neuronal loss and cognitive decline. Additionally, it was observed via PET scan that many individuals have significant amyloid plaque burden without showing any symptoms of memory impairment (Delaere *et al.*, 1990; Dickson *et al.*, 1992; Aizenstein *et al.*, 2008; Villemagne *et al.*, 2011). Furthermore, there is practically no neuronal cell death observed in APP or APP/PS1 mutant transgenic mice modelling FAD with APP related mutations (Ricciarelli and Fedele, 2017). On the other hand, it has been shown, that changes in the distribution patterns of NFTs, NTs and neuronal loss correlate with the severity of AD (Braak and Braak, 1991). Furthermore the reduction of endogenous tau in AD mouse models is able to protect them from Aβ-induced synaptotoxicity and memory deficits (Ittner *et al.*, 2010; Leroy *et al.*, 2012). These results let to the assumption, that Aβ aggregation leads to the aggregation of tau which than causes the actual symptoms of AD (Bloom, 2014). However, there seems to be no temporal or regional correlation in the distribution of NFTs and Aβ plaques in AD patients. In fact the formation of tau tangles actually seems to precede the one of Aβ plaques while the distribution of NFTs correlates way better with the development of clinical symptoms (Price *et al.*, 1991; Schönheit, Zarski and Ohm, 2004). Additionally a recent study showed that the amount of tau tangles, but not Aβ deposits, predicts the rate of subsequent atrophy with the highest correlation of tau deposits and atrophy in younger patients (La Joie *et al.*, 2020).

Impressive preclinical evidence from experiments with AD mouse models as well as patient derived pluripotent stem cells indicates that tau pathology is able to progress independent from Aβ accumulation and downstream of factors like apolipoprotein E, the endocytic system, cholesterol metabolism and microglial activation (van der Kant, Goldstein and Ossenkoppele, 2020).

This is in line with the fact that immunotherapies which target Aβ have failed to halt dementia and only had a minor effect on tau pathology (Nicoll *et al.*, 2006, 2019; Nakagami, 2019).

Overall, the amyloid cascade hypothesis was not able to fully explain the pathomechanisms underlying AD, and the role of Aβ overproduction and deposition in FAD and SAD stays cryptically.

Therefore, the scientific community increased their focus on tau and tauopathies.

1.1.3 Tauopathies

Tau aggregates and builds deposits in neurons and glial cells in more than 20 disorders including AD, progressive supranuclear palsy (PSP), cortico basal degeneration (CBD), Pick's, disease (PiD), postencephalitic parkinsonism (PEP), and frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) (Arendt, Jens T Stieler and Holzer, 2016) These tauopathies have been distinguished into four different classes based on the ratio of the tau isoforms involved in their fibrillary lesions (table1). Some tauopathies contain only 3 microtubule binding repeats (3R), some contain 4 microtubule binding repeats (4R) and some contain both (3R and 4R) (see also 1.2.1). Most of these diseases occur sporadic (V M Lee, Goedert and Trojanowski, 2001; Virginia M-y Lee, Goedert and Trojanowski, 2001; Arendt, Jens T. Stieler and Holzer, 2016). While it was shown that tau dysfunction is clearly able to cause neurodegeneration, and some of the tauopathies show mutations of the tau gene MAPT (see also 1.2.2), it stays poorly understood via which mechanisms tau is involved in these neurodegenerative disorders (Arendt, Jens T. Stieler and Holzer, 2016). The clinical symptoms vary as well between the different tauopathies, depending on the affected brain regions (Williams, 2006). One of the most intriguing questions with high relevance for both, the physiological and pathophysiological function of tau in these context is the large heterogeneity of tau isoforms in the different tauopathies and the biological meaning of these different tau isoforms (Arendt, Jens T. Stieler and Holzer, 2016).

Class I	Class II	Class III	Class IV
tau isoforms 3R & 4R	**predominant 4R**	**predominant 3R**	**Short 0N3R**
AD	Progressive supranuclear palsy (PSP)	Pick's disease	Myotonic dystrophy
Down's syndrome	Corticobasal degeneration (CBD)	Some cases of FTDP-17	
Parkinsonism–dementia complex of Guam	Argyrophilic grain disease (AGD)		
Niemann–Pick disease type C	Globular glial tauopathy (GGT)		
Chronic traumatic encephalopathy (CTE)	Ageing-related tau astrogliopathy (ARTAG)[a]		
Some cases of FTDP-17	Some cases of FTDP-17		

[a] Clinical significance is uncertain and awaits further studies.

Table 1: Molecular classification of tauopathies according to the predominant tau isoform pattern.

Based on the ratio of the 3R and 4R tau isoforms involved in the fibrillary lesions, four different classes of tauopathies have been distinguished (Arendt, Jens T Stieler and Holzer, 2016).

1.2 Microtubule-associated protein tau (MAPT)

1.2.1 Tau occurrence and isoforms

Tau proteins belong to the family of the microtubule-associated proteins (MAPs) (Weingarten *et al.*, 1975) They are expressed in many animal species comprising *Caenorhabditis elegans* (Goedert *et al.*, 1996), *Drosophila* (Cambiazo, González and Maccioni, 1995), rodents (Lee, Cowan and Kirschner, 1988), bovines (Himmler, 1989), goat (Nelson *et al.*, 1996), monkeys (Nelson *et al.*, 1996), and humans (Goedert *et al.*, 1989; Nelson *et al.*, 1996).

In humans the tau gene is located on chromosome 17 at band position 17q21 (Neve *et al.*, 1986). Tau is expressed in the brain in neurons, astrocytes and oligodendrocytes (Shin *et al.*, 1991).

It is also expressed in neurons of the peripheral nervous system, skeletal and heart muscle tissue, skin, gastrointestinal tract, lung, kidney, ovary, pancreas, fibroblasts and lymphocytes (Iis, 1996; Thurston, Zinkowski and Binder, 1996; Miklossy *et al.*, 1999; Pryor *et al.*, 2002; Askanas and Engel, 2008; Makrantonaki *et al.*, 2012; Dugger *et al.*, 2016).

The human tau gene contains 16 exons (Andreadis, Broderick and Kosik, 1995). The primary transcript of tau contains 13 exons, as the exons 4A, 6 and 8 are not transcribed in human. The Exons −1 and 14 are transcribed, however not translated. Exons 1, 4, 5, 7, 9, 11, 12, 13 are always translated, while the exons 2, 3, and 10 are alternatively spliced. In consequence there are six different tau isoforms (figure1). These isoforms differ by the absence or presence of exon 2 and 3 in the amino-terminal part, in combination with either three or four repeat-regions (R1, R3 and R4 or R1–R4) in the carboxy-terminal part and absence or presence of exon 10 in the carboxy-terminal part. The shortest tau isoform (missing exon 2,3 and 10) is only found in the fetal brain and is therefore referred as fetal tau isoform (Buée *et al.*, 2000).

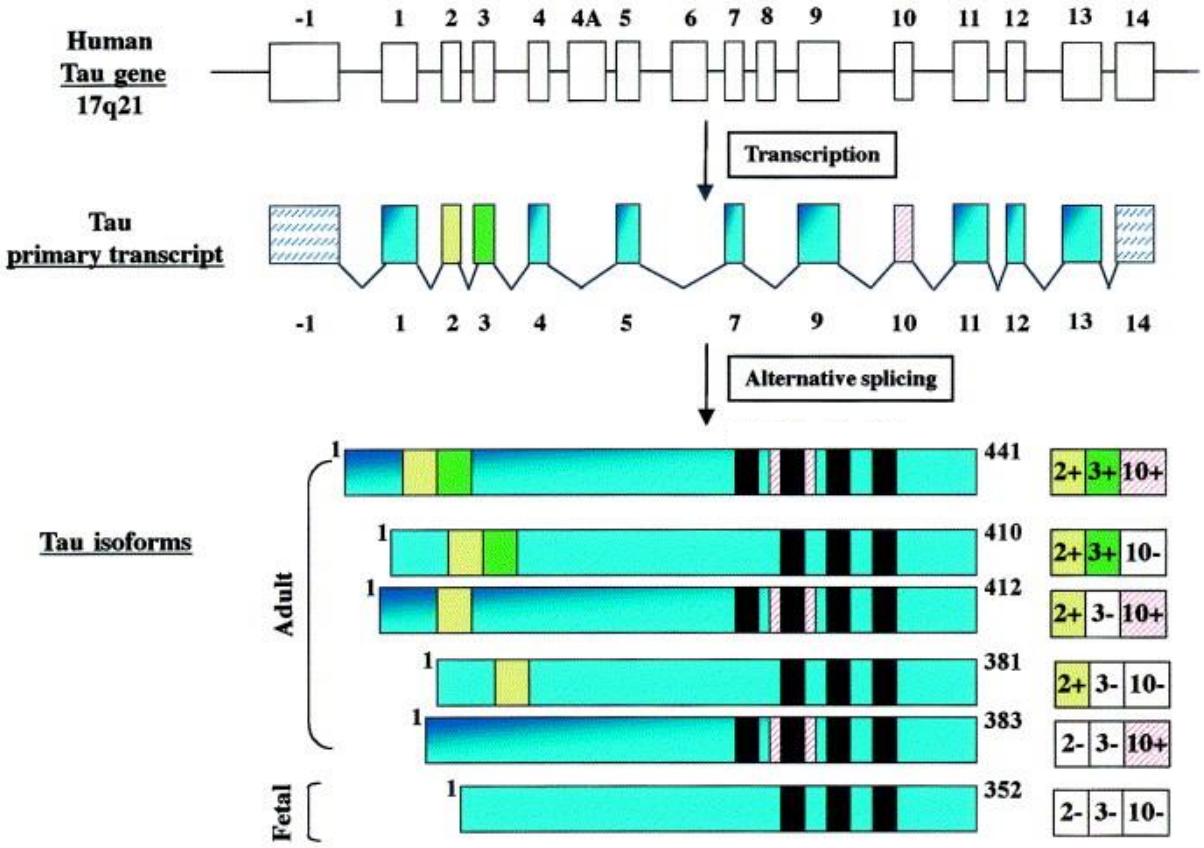


Figure 1: Tau Isoforms

The human tau gene is located over 100kb on the long arm of chromosome 17 at position 17q21. It contains 16 exons, exon −1 is a part of the promoter (upper panel). The tau primary transcript contains 13 exons, since exons 4A, 6 and 8 are not transcribed in human (middle panel). Exons −1 and 14 are transcribed but not translated. Exons 1, 4, 5, 7, 9, 11, 12, 13 are constitutive, and exons 2, 3, and 10 are alternatively spliced, giving rise to six different mRNAs, translated in six different CNS tau isoforms (lower panel). These isoforms differ by the absence or presence of one or two 29 amino acids inserts encoded by exon 2 (yellow box) and 3 (green box) in the amino-terminal part, in combination with either three (R1, R3 and R4) or four (R1–R4) repeat-regions (black boxes) in the carboxy-terminal part. The fourth microtubule-binding domain is encoded by exon 10 (slashed box) (lower panel). The adult tau isoforms include the longest 441-amino acids component (2+3+10+), the 410-amino acids component (2+3+10−), the 412-amino acids component (2+3−10+), the 381-amino acids component (2+3−10−) and the 383-amino acids component (2−3−10+). The shortest 352-amino acids isoform (2−3−10−) is found only in the fetal brain, and thus is referred as fetal tau isoform (Buée *et al.*, 2000).

1.2.2 Tau Mutations

Most tauopathies occur sporadic whiteout any detectable mutations as the cause of disease (Lee, Goedert and Trojanowski, 2001; Arendt, Jens T. Stieler and Holzer, 2016). Disease causing mutations of the tau gene MAPT are normally associated with FTDP-17 (Hutton *et al.*, 1998; Poorkaj *et al.*, 1998). Thus far 53 mutations causing FTDP-17 have been found (Ghetti *et al.*, 2015). The majority of the mutations are located between exons 9-13 in the microtubule- binding domain and in intron 10 (Rademakers, Cruts and Van Broeckhoven, 2004; Ghetti *et al.*, 2015). Mutations between exons 9-13 predominantly lead to tau filament deposits in neurons. Mutations in intron 10 can cause tau deposition in neurons and glial cells (Ghetti *et al.*, 2015). In addition to FTDP-17, MAPT mutations have been found for some cases of Pick's disease, PSP, CBD, and GGT (Murrell *et al.*, 1999; Hogg *et al.*, 2003; Tacik *et al.*, 2015).

Due to an inversion polymorphism on chromosome 17q21.31 there are two haplotypes auf tau gene MAPT, MAPT H1 and MAPT H2. There are multiple H1 sub haplotypes but only one H2 haplotype (Stefansson *et al.*, 2005). The H2 haplotype protects against PSP, while inheritance of H1 is a risk factor for PSP and CBD (Williams and Lees, 2009). However, there are no obvious pathogenic missense or splice site mutations in MAPT in the large majority of sporadic cases of PSP and CBD (Ghetti, Wszolek and Boeve, 2011). A direct connection between MAPT mutations and AD has not been proven so far.

1.2.3 Tau functions

Tau promotes the assembly and stabilization of microtubules (MTs) (Lee and Rook, 1992). It plays an important role in neurogenesis, axonal maintenance and axonal transport (Hernandez and Avila, 2007). Tau is predominantly present in the cytoplasma but it can also be found associated to the cell membrane and the nucleus (Arrasate, Pérez and Avila, 2000; Maina, Al-Hilaly and Serpell, 2016).

The functions of tau depend on the different tau domains.

The N-terminal domain is involved in the regulation of MT dynamics by influencing the attachment between MTs and other cell components (Chen *et al.*, 1992). It inhibits axonal transport in neurons (Kanaan *et al.*, 2011) and influences the subcellular localization of tau (Liu and Götz, 2013; Paholikova *et al.*, 2015). Additionally, the N-terminal region mediates the interaction of tau with the plasma membrane and different proteins like apolipoprotein A1, β-synuclein and synaptophysin (Brandt, Léger and Lee, 1995; Liu *et al.*, 2016).

The proline-rich domain seems to play a role in modulating the signaling functions of tau via binding of several protein kinases (Morris *et al.*, 2011). Additionally, the proline-rich domain regulates the interaction of tau with DNA and RNA (Wang *et al.*, 2006; Qi *et al.*, 2015).

Besides that the proline-rich domain is involved in the regulation of MT assembly and actin binding of tau (He *et al.*, 2009; Maina, Al-Hilaly and Serpell, 2016).

As the name suggests, the microtubule binding repeats (R1-R4) mediate the binding of tau at the microtubules. The flanking sequences, which separate the repeats from each other, play a regulatory role in this process (Mukrasch *et al.*, 2007; Sillen *et al.*, 2007). Other proteins which bind to the repeat domain are α-synuclein (Jensen *et al.*, 1999), histone deacetylase 6 (HDAC6) (Ding, Dolan and Johnson, 2008), apolipoprotein E (Huang *et al.*, 1995), PS1 (Takashima *et al.*, 1998) and F-actin (Correas, Padilla and Avila, 1990). By binding F-actin and MTs through the repeat domain tau acts as an important molecular tether between the actin and microtubule cytoskeleton (Elie *et al.*, 2015). Additionally, the repeat domain binds to lipid membranes, DNA and RNA (Wang *et al.*, 2006; Georgieva *et al.*, 2014; Qi *et al.*, 2015).

For the C-terminal region function and binding partners are not known.

Besides tau there are some other MT associated proteins (MAPs) with structural and functional similarities like MAP2 and MAP4 (Mandelkow and Mandelkow, 1995; Dehmelt and Halpain, 2005). The MAP1 family shows some functional and structural parallels however differs in the MT binding structures (Halpain and Dehmelt, 2006).

1.2.4 Post-translational modifications of tau

In order to regulate the various functions of tau, a wide range of post-translational modifications are performed on this protein. These modifications include phosphorylation, truncation, isomerisation, glycation, nitration, addition of β-linked N-acetylglucosamine (O-GlcNAcylation), acetylation, oxidation, polyamination, sumoylation, and ubiquitylation (Martin, Latypova and Terro, 2011). An overview is shown below (figure 2).

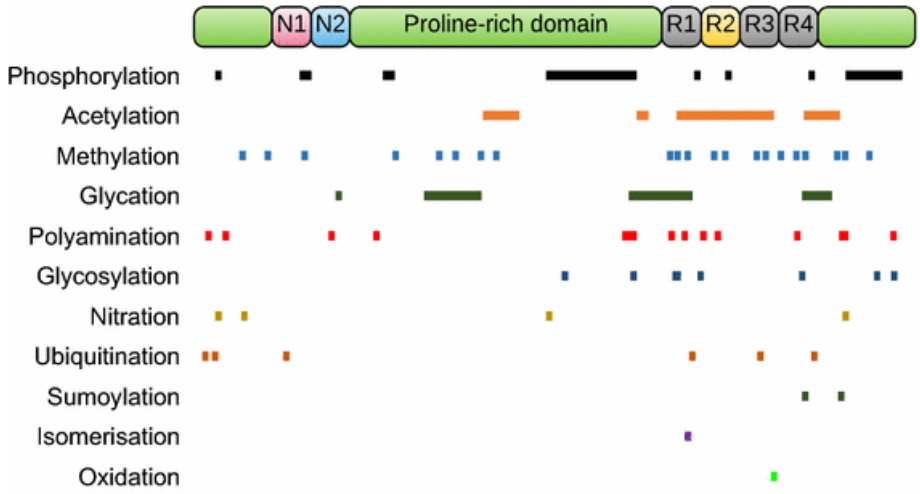


Figure 2: Post-translational modifications of tau.

The coloured bars indicate the approximate sites of each modification on the largest human CNS tau isoform (2N4R, 441 amino acids) (Guo, Noble and Hanger, 2017).

1.2.4.1 Tau phosphorylation

The phosphorylation of tau is the most prominent tau modification, which was studied to the highest extent. This is due to the fact, that tau in NFTs is highly phosphorylated (Goedert *et al.*, 1992). Tau comprises 85 potential phosphorylation sites, respectively 45 serine, 35 threonine, and five tyrosine residues (Hanger, Anderton and Noble, 2009). With phosphorylated tau in NFTs and the large number of potential phosphorylation sides it is no surprise, that tau phosphorylation has a big impact on its physiological function. The increase in tau phosphorylation under pathological conditions reduces its affinity towards MTs which results in destabilization of the cytoskeleton (Drewes *et al.*, 1995). The detached tau itself undergoes self-aggregation resulting in tau oligomers and higher order tau aggregates (Kopke *et al.*, 1993; von Bergen *et al.*, 2000). Additionally, tau phosphorylation alters its interaction with the cytoplasmic membrane, DNA, Fyn and other binding partners, leading to a range of impaired signaling pathways (Hanger, Anderton and Noble, 2009). The phosphorylation and dephosphorylation of tau is regulated by different kinases and phosphatases (Avila, 2008). The kinases can be summarized in three broad groups: (a) proline-directed protein kinases (PDPKs), which are serine/threonine kinases and include glycogen synthase kinase-3 (GSK-3), cyclin-dependent kinase-5 (Cdk5) and mitogen-activated protein kinases (MAPKs), (b) non-PDKs, which are serine/threonine kinases including cAMP-dependent protein kinase A (PKA), casein kinase 1 (CK1) and microtubule affinity-regulating kinases (MARKs), and (c) protein kinases specific for tyrosine residues including Src, Fyn, Abl, and Syk (Martin, Latypova and Terro, 2011).

The phosphorylation patterns of tau first led to the idea that individual kinases are modulating the functions of tau, however many residues are phosphorylated by different kinases and many kinases phosphorylate numerous different residues. The kinases GSK-3, cdk5, CK1 and PKA have been identified as important multi residue kinases for tau and were intensively studied as potential drug targets in AD (Hanger, Anderton and Noble, 2009). For the tau phosphatases, protein phosphatase 2A (PP2A) is responsible for more than 70% of the cellular phosphatase activity in the brain (Liu, Grundke-Iqbal, *et al.*, 2005). PP2A dephosphorylates tau and seems to play a role in the regulation of tau phosphorylation (Gong *et al.*, 2002). Additionally, the activity of PP2A is decreased by ca. 50% in AD brains (Liu, Grundke-Iqbal, *et al.*, 2005). Protein phosphatase 5, (PP5), is another important phosphatase which dephosphorylates tau and is reduced in activity by ca. 20% in AD brains (Liu, Iqbal, *et al.*, 2005).
Together these findings show that hyperphosphorylation of tau is most likely the result of a disturbance in the complex interplay of various kinases and phosphatases in the brain.

The permanent interplay of tau phosphorylation and dephosphorylation is also one of the main reasons why till today it is not well known which phosphorylation sites of tau are involved in the formation of tau pathogenesis and which sites are phosphorylated after the establishment of the pathology.

1.2.4.2 Tau truncation

The truncation of tau occurs under pathological and physiological conditions in various cells and brain tissues. Tau is the target of a huge variety of proteases such as caspases, calpains, thrombin, cathepsins and PSA (puromycin-sensitive aminopeptidase), which cleave it at many distinct proteolytic sites (Hanger and Wray, 2010; Quinn *et al.*, 2018). The role of tau fragmentation for neurodegeneration is still debated. Truncations of tau at position 421 by caspase3 and position 391 by HTrA1 are associated with the formation of NFTs in AD (Basurto-Islas *et al.*, 2008) and cleavage of tau by asparagine endopeptidase (AEP) is believed to promote tau pathology (Zhang *et al.*, 2014). Contrary to these results, in samples from AD patients, full-length tau is the major component of the PHFs (Goedert et al., 1992) and in human mutant tau transgenic P301S or P301L mice tau truncation at position 421 was only found in small amounts in the brain in a late stage of the tauopathy (Lin, Dickson and Sahara, 2011; Zhang *et al.*, 2014). In addition, overexpression of protease PSA inhibited tau induced neurodegeneration in tau transgenic P301L mice and *Drosophila* (Karsten *et al.*, 2006; Kudo *et al.*, 2011). Mice overexpressing a wild-type 3R tau fragment from amino acid 151-421 (Δtau) only showed a mild phenotype. However, mice expressing Δtau together with full length tau developed severe neurotoxicity.

This toxicity was reversible via halting the expression of Δtau and only oligomers of tau but no fibrils were detected in the affected mice (Ozcelik *et al.*, 2016).

1.2.4.3 Tau acetylation

The acetylation of tau occurs via cAMP-response element binding protein (CREB)-binding protein (CBP) (Min *et al.*, 2010) and via auto-acetylation (Cohen *et al.*, 2013). Tau deacetylation is mediated by sirtuin 1 (SIRT1) and HDAC6 (Cook *et al.*, 2014). CBP acetylates tau at several lysine residues located in the MT binding repeats and the proline-rich region, auto-acetylation occurs preferentially at lysine residues located in the MT binding repeats (Cohen *et al.*, 2016). Tau acetylation at different positions is proposed to be responsible for both, protection from AD and to be involved in the development of AD and other tauopathies.

Acetylation of the tau lysine residues 259, 290, 321 and 353 is reduced in AD brains compared to healthy controls. Acetylation at these residues seems to protect tau from increased phosphorylation and decreases tau aggregation (Cook *et al.*, 2014).

On the other hand, acetylation of tau at lysines 174, 274 and 280 was found in the post-mortem brains of AD, PiD, PSP and frontotemporal lobar degeneration-tau (FTLD-tau) patients, and is supposed to be pathological (Irwin *et al.*, 2013; Min *et al.*, 2015). How the pathological changes in tau acetylation occur and if they can be corrected is still unknown and could be a potential starting point for the development of new treatment strategies against AD.

1.2.4.4 Tau glycosylation

N-glycosylation of tau was only found in AD brains but not in healthy controls (Wang, Grundke-Iqbal and Iqbal, 1996). It is suggested, that N-glycosylation contributes to tau hyperphosphorylation by facilitating phosphorylation and suppressing dephosphorylation (Liu *et al.*, 2002). Why and how it comes to N-glycosylation of tau in AD is unclear.

O-linked N-acetylglucosamine (O-GlcNAc) occurs at serine and threonine residues and might protect against tau phosphorylation, as it takes place at the same amino acids (Liu *et al.*, 2004). It additionally seems to reduce tau aggregation without changing its ability to bind and to stabilize microtubules (Yuzwa *et al.*, 2014). It has also been shown, that tau O-GlcNAcylation and the activity of the responsible protein O-GlcNAc transferase are reduced in the brain of AD patients (Liu *et al.*, 2004; Wang *et al.*, 2016). Therefore, O-GlcNAc regulation could be a promising target for potential AD therapy.

For the remaining post-translational modifications, the relevance for tau function and toxicity is not well investigated by now.

Deamination of tau at asparagine residue 279 (N279) takes place in AD at the 4-repeat isoform, but not in CBD or PSP, and might reduce the ability of tau to bind MTs (Dan *et al.*, 2014).

Tau isomerization by Pin1 seems to play a role in tau dephosphorylation at Thr231, and Pin1 dysfunction might play a role in tau pathology (Hamdane *et al.*, 2006).

Tau nitration caused by nitrative injury could be involved in the development of filamentous tau inclusions (Horiguchi et al., 2003).

Glycation of tau, in postmortem samples from AD patients, was found at lysine residues with a preference for lysine residues inside the MT binding repeats. This might have an effect on the MT binding ability of tau (Ledesma, Bonay and Avila, 1995).

Tau ubiquitination is regulated by the ubiquitin ligase CHIP, which directly interacts with the heat shock proteins Hsp70/90. Therefore, CHIP leads to tau degradation via ubiquitination. However, CHIP also increases tau aggregation (Petrucelli *et al.*, 2004). Interestingly 73% of the lysine residues targeted by ubiquitination are also the target of acetylation, indicating an interplay of acetylation and ubiquitination in tau regulation (Morris *et al.*, 2015).

Tau sumoylation has its mayor target site at lysine 340 and counteracts the effects of ubiquitination, while reciprocally stimulating tau phosphorylation, at least in cell culture (Luo *et al.*, 2014).

Tau methylation occurs on lysine and arginine residues. The lysine methylation seems to occur predominantly in the microtubule-binding repeat region and potentially has an effect on tau MT binding and tau aggregation (Yang and Seto, 2008; Funk *et al.*, 2014).

These results show that there are at least four tau modifications which potentially compete for lysine residues (glycation, acetylation, ubiquitination, and methylation). This indicates a strategic role of lysine modification when modifying tau function.

Altogether there are many post-translational modifications of tau, which can be present under physiological and / or pathological conditions and can take place at various sites of tau mediated by a variety of enzymes. This huge amount of variables makes it very difficult to identify which post-translational modifications of tau have the highest impact on health and disease and would be the most promising targets for a controlled adjustment of tau regulation.

1.2.5 Murine tau

The longest isoform of tau in mice and humans has an identity of 89% in the amino acid sequence (Hernández, Merchán-rubira and Vallés-saiz, 2020). The biggest difference between human and murine tau are the N-terminal residues 17 to 28 which are present in human but not in murine tau, the MT binding repeats on the other hand only differ by three amino acids (Hernández *et al.*, 2019). In mouse brain the composition of tau isoforms transitions from the smallest tau isoform (3R-0N) in the fetal brain to a mixture of three 4-repeat isoforms (4R-0N, -1N, -2N) in the adult brain (Kampers *et al.*, 1999). *In vitro* murine tau assembles into Alzheimer-like PHFs with a similar efficiency than human tau (Kampers *et al.*, 1999). The formation of murine tau fibrils can be induced by hyperphosphorylation and the resulting fibrils resemble the ones obtained by hyperphosphorylation of human tau (Chohan *et al.*, 2005). However *in vivo*, mice expressing mTau never develop NFTs even when endogenous mTau is overexpressed (Adams *et al.*, 2009).

1.3 Prions and the prion-like properties of tau

The word prion is derived from proteinaceous and infectious (Prusiner, 1982). Prion diseases (PDs) can occur as genetic, infectious or sporadic disorders. Creutzfeldt-Jakob disease (CJD) shares most symptoms of a progressive dementia, while scrapie of sheep and BSE can be described as ataxic illnesses (Hope *et al.*, 1988). All PDs in mammals involve modifications of the prion protein (PrP), a small, cell-surface glycoprotein, which is expressed under physiological conditions in mammalian cells (Prusiner, 1991). PrP becomes misfolded and aggregates into β-sheet structured amyloid (PrPSc) (Scheckel and Aguzzi, 2018). Tau also develops β-sheet structured amyloids which spread throughout the brain in a specific fashion that is distinct for each tauopathy (Arnold *et al.*, 2013).

Prions spread by forcing their pathological conformation on physiologically folded proteins, thereby transforming them to new prion proteins. The prion spreading seems to appear in a circle of nucleation and fragmentation and can be induced via the introduction of misfolded protein as seeds to start the process (figure3) (Scheckel and Aguzzi, 2018).

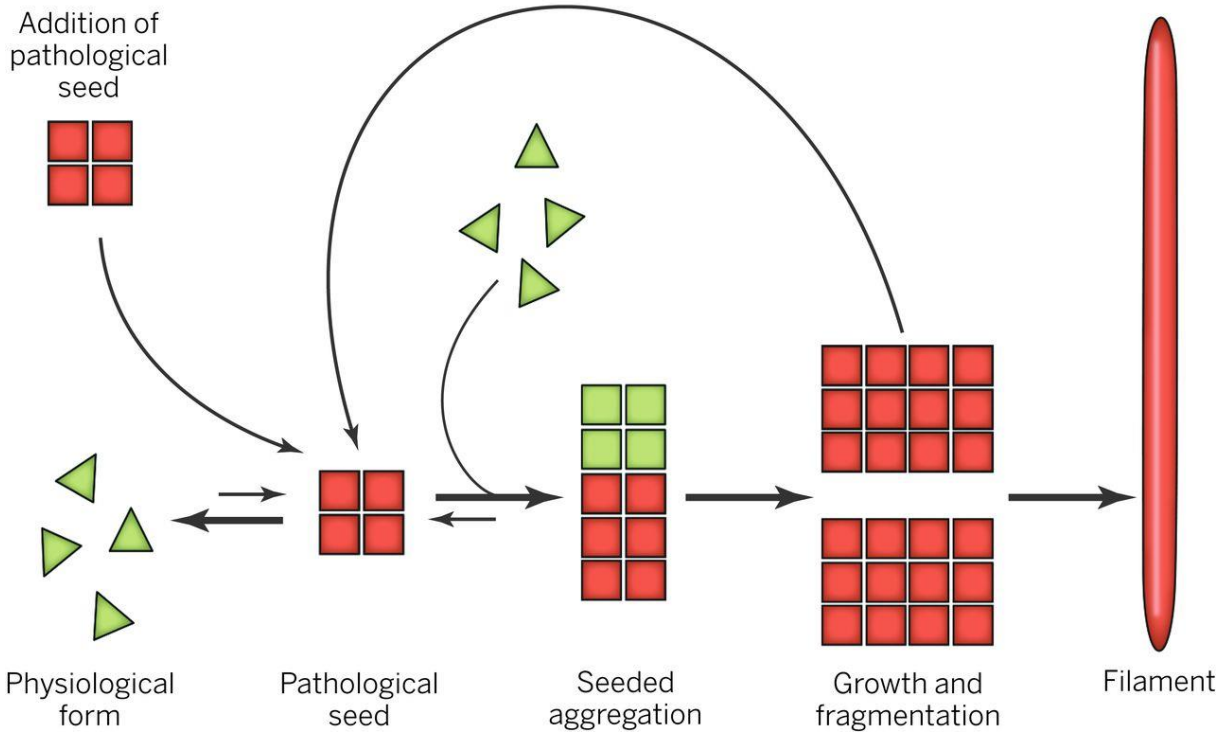


Figure 3: The pathological pathway leading from soluble proteins to insoluble filaments

The formation of pathological seeds is a rare and energetically unfavorable event. Once a seed has formed, single molecules can change shape and join the growing aggregates. Seed addition induces rapid assembly of the soluble protein. Fragmentation generates new seeds, accelerating the formation of aggregates. Filaments represent the endpoints of aggregation (Goedert, 2015).

In case of tau, seeding with different tau seeds leads to the formation of fibrils with different conformations. These fibrils are able to keep their specific conformations and biochemical properties throughout several passages *in vivo* as well as *in vitro* (Kaufman *et al.*, 2016). Other proteins which can be seeded in a pathological conformation and then spread through the brain are the second hallmark protein of AD Aβ and α-synuclein in Parkinson disease (Musiek and Holtzman, 2015; Kim *et al.*, 2019). While these proteins share the same mechanisms of misfolding and propagation their infectivity seems to be lower than the one of PrP. Therefore the term prionoids has been proposed for these neurodegenerative proteins (Scheckel and Aguzzi, 2018).

1.3.1 Yeast prion Sup35 as a potential tau seed

The most simplistic eukaryotes in which prions occur are yeast, which are single cell organisms. The yeast prion that was first discovered and most studied is Sup35 (Wickner, 1994). It is composed of 3 sequence elements (Paushkin *et al.*, 1997), a N-terminal (N), a middle (M) and a C-terminal (C) domain (Lindquist *et al.*, 2004). In yeast Sup35 acts as a translation termination factor, which reacts to a lowering of the pH via liquid-like phase separation and the formation of reversible protective gels. This process is regulated via the N domain of Sup35 (Franzmann *et al.*, 2018). Prion forms of Sup35, called [PSI+], are found in many wild yeast strains and in *Saccharomyces cerevisiae* strains of commercial baker's yeast, brewery yeast and dry wine yeast (Halfmann *et al.*, 2012; Kelly, Busby and Wickner, 2014). Like tau and PrP, Sup35 aggregates into β-sheet structured amyloid. The N and M domain are sufficient to form Sup35NM fibrils harboring prion properties (Gorkovskiy *et al.*, 2014). Sup35 prions as well show the remarkable stability observed in prion amyloid assemblies of prions like PrP, which enables them to survive the transfer from one organism to another (Halfmann *et al.*, 2012; Walker *et al.*, 2013). Furthermore, the Sup35NM domain was shown to propagate as a prion in mammalian cells (Krammer *et al.*, 2008). Tau and Sup35 are both intrinsically soluble, disordered and unfolded proteins, which can form highly ordered β-sheet structured amyloid aggregates closely resembling each other in case of tau and Sup35 fibrils composed of fibril-forming segments from the respective protein (Sawaya *et al.*, 2007; Mirbaha *et al.*, 2018; Ohhashi *et al.*, 2018). In this context it is interesting to mention, that long before the knowledge of prionoids and the transcellular spreading of tau it was proposed, that an infectious agent enters the cranium and brain from the nasopharyngeal cavity and spreads from there via the structures closely linked with each other by neuronal pathways (Ulrich, 1985). Additionally, in a recent article it was shown, that gut injection of α-synuclein fibrils leads to the conversion of endogenous α-synuclein to a pathological form which travels to the brain via the vagus nerve and causes features of Parkinson disease (Kim *et al.*, 2019).

Based on these discoveries and in light of the recently growing understanding of the human microbiome (Gilbert *et al.*, 2016) it is desirable to investigate if an archaic natural yeast protein like Sup35 is able to cross-species cross-seed a human neurodegenerative prionoid like tau.

1.3.2 Other yeast prions

Ure2 is another yeast prion which is able to build amyloid fibrils *in vivo* and *in vitro* and plays an important role in nitrogen catabolism (Baxa *et al.*, 2002). The prion form of Ure2 is unable to block the uptake of ureidosuccinate therefore delivering a route for the synthesis of Uracil in environments lacking this important nucleobase (Wickner, 1994). Other than Sup35 aggregates, Ure2 oligomers and fibrils are toxic to mammalian cells (Pieri *et al.*, 2006). Interestingly Ure2 and Sup35 do not show amino acid identity with each other or with mammalian PrP (Tuite, 2000). While the prion-forming domains of both proteins do not share significant amino acid sequence identity they do share an atypically high proportion of the polar amino acids Gln and Asn, which contain uncharged R groups, as well as an overall low charge content (Tuite, 2000). Another yeast prion with a Gln+Asn-rich prion-forming domain is Rnq1. While the function of the Rnq1 prion [RNQ+] in *Saccharomyces cerevisiae* is unknown [RNQ+] is able to promote the formation of the Sup35 prion besides being completely non-homologous (Derkatch *et al.*, 2004; Westergard and True, 2014). It was therefore hypothesized that [RNQ+] primes the cell for the formation of the Sup35 prion in order to induce it in conditions where Sup35 prion formation can be advantageous (Westergard and True, 2014). Together this data shows the existence of a variety of prions in yeast which highly defer in their respective DNA sequence. This gives evidence that structural similarities are more important for prion seeding than similarities in the DNA assembly.

1.3.3 Prions in bacteria, plants and viruses

Besides mammals and yeast, prion proteins have also been discovered in bacteria, plants and viruses (Chakrabortee *et al.*, 2016; Pallarès, Iglesias and Ventura, 2016; Yuan and Hochschild, 2017; Nan *et al.*, 2019). For bacteria it was discovered that the protein curli, which is expressed by *E. coli* and other Enterobacteriaceae, aggregates and builds amyloid fibers which are a central component of their extracellular matrix and therefore play a key role for their environmental persistence (Chapman *et al.*, 2002; Smith *et al.*, 2017). Additionally, the Rho termination factor of *Clostridium botulinum* (Cb-Rho) contains a prion-like domain with an amyloidogenic core, which enables Cb-Rho to form amyloid fibrils (Pallarès, Iglesias and Ventura, 2016; Yuan and Hochschild, 2017).

For plants the prion-like domain of Arabidopsis protein Luminidependens (LDPrD) was able to form higher-order oligomers and was capable of replacing the prion-domain functions of Sup35 in yeast (Chakrabortee *et al.*, 2016).

For viruses, protein LEF-10 behaves as a prion in Autographa californica multiple nucleopolyhedrovirus (AcMNPV), a large double-stranded DNA virus that infects insects. In case of high multiplicity of infection in virus-infected cells LEF-10 can change its conformation to an aggregated state, which leads to the inhibition of viral late gene expression (Nan *et al.*, 2019).

These findings underline the far-reaching prevalence of prion proteins in living organisms from many different species. Furthermore, these results suggest, that the development of prions happened a very long time ago as these mechanisms are so widespread in nature.

1.4 Prion-like behavior of tau in animal models for tauopathies

1.4.1 Induction of tau aggregation by expression of mutated tau

Several mouse lines were developed with the aim to study tau pathology *in vivo*. These mouse lines express human tau (htau) that carries MAPT mutations associated with FTDP-17 like G272V, P301L, P301S, V337M and R406W (Frank, Clavaguera and Tolnay, 2008). In contrast to mice which express wild type (WT) htau most of these mice, expressing pathological tau mutants, develop NFTs and NTs several months after birth. Additionally, these mice develop neuronal cell loss which manifests in motoric and cognitive impairments.

One of these mouse models which was extensively studied is the P301S htau mouse model (P301S mouse) (Allen *et al.*, 2002). P301S mice express the shortest 4R htau isoform (0N4R, 383 aa) comprising the P301S mutation (proline replaced by serine at position 301 of the amino acid sequence). The expression of the P301S transgene is controlled by the *thy-1.2* promoter which has the highest expression rates in neurons of the brain stem and the spinal cord. The overexpressed P301S tau in these mice is hyperphosphorylated and fibers can be found in nerve cells in brain and spinal cord. Homozygous mice show a 49% reduction in the number of motor neurons of the spinal cord, likely being the main cause for the paraparesis, muscle weakness and tremor that mice develop at the age of ca. 6 months.

1.4.2 Induction of tau aggregation by inoculation of seeds

The development of NFTs can be induced by intracerebral injection of tau seeds (Clavaguera *et al.*, 2009). The injection of Alz17 mice, which express the longest human tau isoform and do not exhibit filamentous tau aggregates (Probst *et al.*, 2000), with brain homogenates of P301S mice containing tau fibrils, leads to tau aggregation spreading from the injection site (Clavaguera *et al.*, 2009). After the injection the Alz17 mice developed hyperphosphorylated htau, NFTs and NTs and coilded-bodies in a progressive manner. The depletion of the homogenates from htau abolished this seeding effect. The induced changes of tau confirmation spread to connected brain regions with an increase in reach over time and a higher spreading radius for hyperphosphorylated htau compared to NFTs and NTs. It is important to note, that the injected Alz17 mice did not develop neuronal loss or any of the motoric impairments present in old P301S mice. The lack of motoric impairment most likely comes from the fact that the fibrils were inoculated in the hippocampus of the mice (Clavaguera *et al.*, 2009) while motoric problems result from the death of neurons in brain stem and spinal cord (Allen *et al.*, 2002). As tau spreading is a slowly advancing process and the hippocampus is only remotely connected to the brain stem the mice likely just dye of old age before the spread of tau aggregation reaches the brain stem. This estimation is in accordance with the fact, that in cases where human patients where accidently infected with PrPSc or Aβ, by contaminated growth hormones, it took an incubation time exceeding 30 years until neurodegeneration was observed (Jaunmuktane *et al.*, 2015; Purro *et al.*, 2018). In another seeding trial injection of presymptomatic 2 months old P301S mice with brain homogenate of symptomatic P301S mice led to accelerated tangle formation with increased density of hyperphosphorylated htau (Ahmed *et al.*, 2014).

Besides mouse derived tau fibers, tau extracts from AD patients and tau filaments generated *in vitro* also have the ability to induce hyperphosphorylation and aggregation of tau in different mouse models (Clavaguera *et al.*, 2013; Sanders *et al.*, 2014; Kaufman *et al.*, 2016). The brain homogenates from post mortem patient biopsies of various tauopathies including AD have the ability to induce the aggregation of WT htau in Alz17 mice.

Remarkably filaments formed in the brains of the injected mice have the same confirmation as the tau fibers from the respective tauopathy patients. Furthermore, tau aggregates collected from mice seeded with patient samples and reinjected in new mice, again have the same structure as the original tau structures observed in the respective tauopathy patients (Clavaguera *et al.*, 2013). *In vitro* generated tau fibers as well kept their specific conformation when seeded and reseeded for two times. This was the case for seeding in mice via brain injection as well as for *in vitro* seeding in cells (Sanders *et al.*, 2014).

On top of that the generation of very distinct tau fibers *in vitro* and their injection in mutant tau expressing P301S mice resulted in a strain specific progression and spreading of tau pathology.

The P301S mice showed different progression rates and different affected brain regions concerning abnormal tau phosphorylation (Kaufman *et al.*, 2016).

1.5 Cryo-EM structure of tau filaments from AD, Pick's disease, Chronic traumatic encephalopathy (CTE) and CBD

In recent years, scientist have been able to resolve the structures of various tau filaments from different tauopathies. They discovered that PHFs and straight fibrils of tau had the same C-shaped protofibril structure in post mortem samples of an individual with AD (Fitzpatrick *et al.*, 2017). The filaments of Pick's disease tau consist of residues Lys254–Phe378 and are folded differently than the filaments in AD (Falcon *et al.*, 2018).

The observed structure of tau folding in tau from a post mortem Pick's disease patient explained the selective aggregation of 3R tau and the difference in phosphorylation compared to the tau filaments found in the samples of an AD patient (Falcon *et al.*, 2018). In CTE, similar to AD, 3R and 4R tau aggregate in order to form C-shaped protofilaments (Falcon *et al.*, 2019). In CBD tau filaments consist only of 4R tau. CBD fibrils were analyzed by to different groups of scientist, (Zhang *et al.*, 2019; Arakhamia *et al.*, 2020), both groups found in essence the same β-sheet configuration and fold for CBD fibrils. Zhang additionally detected a molecule inside the fold of the protofibril which was not covalently bound and has an estimated negative net charge. Arakhamia on the other hand found post translational modifications (PTMs) of CDB fibrils via mass spectrometry. Interestingly this analysis showed that acetyl and ubiquitin groups predominated in the fibril-forming core of the fibril. If the hydrophilic molecule detected inside the fold of the tau fibril or the PTMs of CDB tau play a role in the folding, and therefore the confirmation, of these tau fibrils still needs to be investigated. For the latter it would be possible to remove PTMs from tau fibrils before using them as seeds and to compare the conformation of the resulting fibrils with the one of fibrils which were seeded by the use of tau seeds containing PTMs.

Together these results suggest that the different tau confirmations specific for different tauopathies play a crucial role in the progression of pathology and the brain areas which are affected by the respective disease.

1.6 Neurotoxicity of tau oligomers in the absence of tau fibrils in a murine tauopathy model

Native tau is a soluble and unfolded protein acting as a microtubule stabilizer in neuronal cells. Previous research suggests, that different modifications of tau including phosphorylation, fragmentation, and conformational changes, can induce filamentous tau aggregation (Sahara, Maeda and Takashima, 2008). This aggregation starts with the formation of oligomeric species and subsequent aggregation into fibrils, culminating in the formation of tangles. Tau aggregation can spread towards anatomically connected regions in a prion-like manner (Clavaguera *et al.*, 2009; Goedert, Eisenberg and Crowther, 2017). In our lab it was recently shown that tau toxicity can be mediated by oligomeric tau species (Ozcelik *et al.*, 2016).

If heterozygous P301S mice are crossed with Tau62 mice, expressing the 3R tau151–421 (Δtau) fragment, the co-expression of full-length P301S mutant tau with Δtau in 3-weeks-old P301SxTAU62 transgenic mice leads to the formation of soluble high molecular weight tau oligomers. These oligomers are sufficient to cause extensive nerve cell dysfunction and severe motor palsy in the absence of insoluble tau aggregates and neurofibrillary tangles (NFT) (Ozcelik *et al.*, 2016). Strikingly, once the doxycycline-inducible expression of Δtau is switched off in these mice, their severe phenotype reverses within 3 weeks, and animals regain their motor competence, even though heterozygous P301S mutant tau expression is maintained (Ozcelik et al., 2016).

1.7 The human microbiome

The human body has co-evolved with trillions of microbes which build ecosystems specific for the body site they inhabit. These communities are collectively referred to as the human microbiome (Methé *et al.*, 2012). As the relationship between microbiota and the host organism has been formed during a long process of co-evolution it is in general symbiotic (Bäckhed *et al.*, 2005; Littman and Pamer, 2011).

Changes in the composition of the microbiota can lead to dysbiosis, which is associated with several diseases such as type 1 and type 2 diabetes, metabolic syndrome, non-alcoholic fatty liver disease and inflammatory bowel disease (Zhang and Zhang, 2013; Nagpal, Yadav and Marotta, 2014; Palm, de Zoete and Flavell, 2015; Knip and Siljander, 2016).

This negative deviation in the gut often leads to the overgrowth of pathogenic bacteria or fungi accompanied with a loss of microbial diversity and an inflammatory response by the host that involves the induction of inflammation mediating T-helper cells like Th1 and Th17 cells (Gaboriau-Routhiau *et al.*, 2009; Ivanov *et al.*, 2009).

Microbiomes often differ to a large degree between different individuals even in the absence of a disease (Huttenhower *et al.*, 2012). The reasons for this interpersonal diversity are mainly unexplained so far, although diet, environment, host genetics and early microbial exposure seem to play a certain role (Qin *et al.*, 2010; Huttenhower *et al.*, 2012). Even shared types of bacteria differ by more than an order of magnitude between healthy individuals (Turnbaugh *et al.*, 2007; Huttenhower *et al.*, 2012). Therefore, it became clear, that it is not practicable to define an ideal set of specific microbes as the composition of a healthy microbiome (Bäckhed *et al.*, 2012; Shafquat *et al.*, 2014). Which circumstances are necessary to enable the establishment and maintenance of a healthy microbiome still has to be investigated.

1.7.1 The potential role of the microbiome in neurodegeneration

The walls of the gastrointestinal tract are innervated by intrinsic and extrinsic neurons regulating many of the functions of the gut. For that reason, the brain is directly connected to the gut via parasympathetic extrinsic neurons in the brainstem and sacral spinal cord which run through the vagus and pelvic nerves (Uesaka *et al.*, 2016). Therefore, Braak and coworkers formed the hypothesis, that the initial induction of α-synuclein aggregation is caused by a yet unknown agent in the gut which enters the brain via the vagus nerve (Braak *et al.*, 2003). Indeed, in Parkinson the same α-synuclein deposits are found in the gut and in the brain (Braak et al., 2004). Additionally, it was shown, that in mice exogenous misfolded α-synuclein in the gut leads to misfolded α-synuclein in the brain via the vagus nerve (Kim *et al.*, 2019). However, the results from Parkinson patients are less clear, while a Swedish study found a small protective effect for vagus nerve dissection starting 5 years post-surgery (Liu et al., 2017) a Danish study on the same topic was not able to find any significant difference between patients and controls with an intact vagus nerve (Tysnes et al., 2015). This suggests, that the vagus nerve is not the only route which can be used by α-synuclein in order to enter the brain. In line with this Braak and coworkers proposed a dual hit theory, proposing that a neurotropic pathogen enters the brain via the nose and gut. They suggested the initial process to be nasal. The pathogen is then swallowed in saliva and mucus, which allows it to cross the stomach wall and to invade the distal fibers of the vagus nerve, damaging the vagal dorsal motor nucleus in the medulla.

Simultaneously, the pathogen invades the brain from the nasal mucosa spreading via the olfactory bulb and anterior olfactory nucleus into the olfactory structures of the temporal lobe (Hawkes, Tredici and Braak, 2009). In line with this theory, researchers were able to show that injection of α-synuclein fibers into the olfactory bulbs of WT mice results in olfactory deficits and a spreading of α-synuclein which is reminiscent of the pattern observed in early stages of Parkinson (Rey et al., 2016). For more information about the nasal microbiome and its potential role in the development of neurodegenerative diseases see the text below “The nasopharyngeal microbiota”.

Parkinson patients have an altered microbiome compared to healthy people with a higher presence of pro-inflammatory bacteria (Keshavarzian *et al.*, 2015). *E. coli* are able to produce extracellular amyloid fibers called curli which play a role in the colonization of inert surfaces and the formation of biofilms (Chapman *et al.*, 2002). In line with these results, in a recent study, scientists were able to show that gastrointestinal exposure of mice overexpressing human α-synuclein to bacterial amyloid protein curli leads to increased α-synuclein aggregation in the gut and the brain of those mice (Sampson *et al.*, 2020). Additionally, intravenous injections of curli were able to accelerate amyloid protein A amyloidosis in a mouse model (Lundmark, Westermark and Olse, 2005). An even more potent acceleration of A amyloidosis was achieved by injections of Sup35 fibrils from *Saccharomyces cerevisiae* (Lundmark, Westermark and Olse, 2005). This is additional evidence for the ability of microbiome derived proteins to influence the aggregation of human amyloid formation.

Oral bacteria may also produce amyloid proteins and mutants of the oral symbiont Streptococcus seem to be associated with cerebral micro bleeds (Tonomura *et al.*, 2016). Infection of the oral cavity could thereby allow access to the trigeminal nerve and therefore a connection to the brain (Dando *et al.*, 2014).

1.7.2 The nasopharyngeal microbiota

The nasopharyngeal cavity is densely colonized by a broad variety of microorganisms, many of them are commensals, however the nasopharyngeal cavity is also an anatomical reservoir for pathogenic microbes (Garcia-Rodriguez, 2002). Under certain conditions theses microbes are able to spread to the lower and upper respiratory airways where they cause respiratory infections, or invade the bloodstream, which can lead to sepsis and meningitis (Perez-Losada, A. Crandall and J. Freishtat, 2016; Teo et al., 2016). The nasal sensors are in close contact to the microbiome in the nasopharyngeal cavity and they are anatomically connected with the limbic system, which is also affected early in AD (Ulrich, 1985).

This opens the possibility, that substances like prions from the nasopharyngeal microbiome could travel to the brain via the nasal sensors where they interact with prion like proteins like tau and therefore influence the development of neurodegenerative diseases including AD.

Despite this information and other data about the incidence, prevalence and density of potential pathogens in the nasopharyngeal cavity microbiota of children and adults, the detailed composition of the microbial community in the nasopharyngeal cavity has not been studied yet during health or disease (Bogaert *et al.*, 2011; Proctor and Relman, 2017).

2 Results

2.1 Manuscript No. 1

Cross-seeding of Alzheimer-related prion-like proteins by non-mammalian prions

Martin Flach[1, 2, 5], Cedric Leu[3, 5], Alfonso Martinisi[1, 2], Zhiva Skachokova[1], Stephan Frank[1], Markus Tolnay[1], Henning Stahlberg[3], David T. Winkler[1, 2, 4]

[1]Institute of Medical Genetics and Pathology, and [2]Department of Neurology, University Hospital Basel, Petersgraben 4, CH-4031 Basel, Switzerland, [3] Center for Cellular Imaging and NanoAnalytics (C-CINA), Biozentrum, University of Basel, Mattenstrasse 26, CH-4058 Basel, Switzerland, [4]Neurology, Medical University Clinic, Kantonsspital Baselland, Rheinstrasse 26, CH-4410 Liestal, Switzerland

[5] These authors contributed equally to this work

Correspondence to:

D.T. Winkler, Institute of Pathology and Dept. of Neurology, University Hospital Basel, Petersgraben 4, CH-4031 Basel, Switzerland

Tel: 0041 61 328 61 62; Fax: 0041 61 265 41 00

Email: David.Winkler@usb.ch

Abstract

Objective

Understanding the mechanism provoking tau aggregation in Alzheimer's disease (AD) and other neurodegenerative conditions remains an unmet challenge. We observed that a yeast prion can seed tau protein aggregation. This finding should prompt research efforts aimed at exploring potentially pathogenic roles of non-mammalian prions in neurodegeneration.

Background

AD patient-derived or synthetic tau aggregates can seed unfolded tau into β-sheet structured amyloid in a prion-like manner. Tau can also be cross-seeded by other neurodegenerative prion-like proteins including amyloid-β and α-synuclein. Whereas prions have recently been discovered in a wide range of organisms including bacteria and plants, it remains unclear, whether such non-mammalian prions can cross-seed tau aggregation.

New/Updated Hypothesis

We hypothesize that non-mammalian prions showing similar β-sheet structure as tau aggregates can seed tau aggregation. Such prions might serve as a pathogenic factor in sporadic tauopathies, including AD. In our present study, the yeast Sup35NM prion domain promoted the formation of morphologically distinct tau fibril strains *in vitro*. *In vivo*, hippocampal inoculation of Sup35NM fibrils accentuated tau pathology in P301S tau transgenic mice, providing first evidence for heterotypic cross-species seeding by a non-mammalian prion.

Major Challenges for the Hypothesis

While our study demonstrates seeding of a neurodegenerative prion-like protein by a yeast prion, it remains to be investigated whether such cross-species cross-seedings have any role in the context of neurodegenerative conditions such as AD. Future work should therefore explore potential heterotypic cross-seeding of tau by non-mammalian prions, including bacterial and plant prions. In parallel, cross-seeding of AD associated amyloid-β by non-mammalian prions should be assessed.

Algorithm-based screenings for microbiome-associated non-mammalian prions should be intensified. Eventually, clinical studies should explore the presence of such prions in AD patients.

Linkage to Other Major Theories

Template-based seeding as a nucleation-dependent process in prion aggregation has initially been described by Jarrett and Lansbury. We here extend this concept towards cross-species cross-seeding of seemingly unrelated proteins with prion-like capacities in the context of AD. Our hypothesis builds on previous theories suggesting that in neurodegenerative diseases including AD, an exogenous pathogen might enter the brain via the olfactory system to trigger neurodegeneration.

Keywords

Alzheimer’s disease; neurodegeneration; tau; Sup35; prion; seeding; yeast

1. Objective

This article proposes that non-mammalian prions can be involved in the induction of pathological tau aggregation. This proposition is based on our observation that the yeast prion domain Sup35NM can seed fibril formation of the human tau protein *in vitro* as well as *in vivo*. Our finding provides initial evidence that archaic prions may play a role in the not yet understood pathogenesis of Alzheimer's disease (AD) and other tauopathies. Tau protein aggregation in AD might, amongst other mechanisms, be provoked by non-mammalian prions present in the human microbiome or ingested from the outside, from where they may reach the central nervous system via the olfactory and/or vagal nerves.

2. Background

2.1. *Historical evolution*

Tau is a natively unfolded microtubule-binding protein involved in axonal and nucleocytoplasmic transport, and in translation regulation. In AD, tau is hyperphosphorylated and eventually forms intraneuronal inclusions [1]. Besides AD, neuronal or glial tau aggregates are characteristic for more than 20 diseases, summarized under the term tauopathies [1]. The process of tau aggregation via the self-assembly of intrinsically disordered tau into highly ordered β-sheet-rich amyloid remains only partly understood, but is thought to be similar for all tauopathies [2]. In recent years, scientists have been able to resolve the structures of various tau filaments from different tauopathies. They discovered that paired helical filaments and straight fibrils of tau had the same C-shaped protofibril structure in *post mortem* samples of an individual with AD [3]. Of the tauopathy filaments examined so far, the tau folds of chronic traumatic encephalopathy (CTE) and AD are most similar to each other [3, 4]. In contrast, tau filaments derived from Pick's disease patients were found to be folded differently [5].

Similar to other aggregating proteins involved in neurodegenerative diseases, tau has prion-like properties [3, 5, 6]. Structural transformation of soluble proteins into their prion state is generally accompanied by an increase in β-sheet structure [7]. The template-based induction of β-sheet

structure in amyloidogenic proteins is referred to as “seeding” [8]. AD patient-derived or synthetic tau aggregates can seed unfolded tau into β-sheet structured amyloid [1]. Seeding of tau protein monomers can also be provoked by β-sheet structured aggregates of heterologous proteins - a process known as heterotypic or cross-seeding [9, 10]. Seeding and cross-seeding are both thought to require similar folding and binding characteristics of the partnering proteins [11, 12]. Whereas tau aggregates often occur in parallel with aggregates of heterologous β-sheet structured prion-like proteins, e.g. amyloid-β in AD, α-synuclein in Parkinson’s disease, and huntingtin in Huntington’s disease [13], the reasons for these mixed pathologies are not yet fully understood. While factors such as inflammation, disturbed cellular metabolism and others might be involved, cross-seeding of tau has been demonstrated for several prion-like proteins, including α-synuclein [14-17], amyloid-β [9, 18], and islet amyloid polypeptide (IAPP) [19].

2.2. Rationale

The vast majority of tauopathies, including AD, are sporadic diseases. In these disorders, the initial factor provoking the conversion of unfolded tau into β-sheet structured aggregates remains unidentified [1]. Here, we propose that, besides other factors, non-mammalian β-sheet structured protein templates might serve as initial seeds provoking tau aggregation in sporadic tauopathies.

β-sheet structured amyloids are not only associated with a wide variety of neurodegenerative disorders, but they also occur in all forms of life. More than a dozen proteins which form β-amyloid structured prions have been found in fungi [7]. Recently, prions have also been discovered in bacteria [20-22], plants [23, 24], and even in the form of a viral expression factor [25]. The discovery that prions are widely present in nature and show structural similarities with human prion-like proteins like tau opens up the perspective that heterotypic seeding by non-mammalian prions might play a role in the pathogenesis of neurodegenerative diseases. Therefore, we decided to analyze, as a proof of concept, whether an archaic natural yeast prion would be able to seed human tau protein.

For several reasons, we chose the yeast Sup35 prion to study the tau cross-species cross-seeding capacity of a non-mammalian prion. First, Sup35 prions form a parallel in-register β-sheet structured amyloid similar to tau aggregates [26]. Second, several yeast prions are known to promote the

aggregation of other yeast prions, suggesting heterogeneous seeding competence [27, 28]. Moreover, Sup35NM was shown to accelerate amyloid formation in a murine model of silver nitrate-induced serum amyloid A amyloidosis [29]. Third, Sup35 is a naturally occurring prion, so far detected in wild yeast on grapes, in strains of commercial dry wine yeast [30], and in yeast samples from breweries and commercial fruits [31].

Sup35 is the yeast prion first discovered and most studied [32]. The combined N-terminal (N) and middle (M) domains of Sup35 are sufficient to form Sup35NM fibrils with prion properties [33]. Similar to tau, Sup35 is involved in phase separation [34, 35]. Sup35NM is highly aggregation prone and only stays monomeric when purified in denaturing conditions, which require a high concentration of reducing agents [36-39]. Therefore, recombinant tyrosine-deleted Sup35NM protein (-5TyrSup35NM), previously described as less aggregating [32], was used as a non-aggregated seeding control for our *in vitro* and *in vivo* seeding experiments.

3. New or updated hypothesis

We hypothesize that non-mammalian microbiotic or nutritional prions might, amongst others, serve as an aggregation-inducing pathogenic factor in sporadic tauopathies, including AD. As a proof of concept, we aimed for cross-seeding of human tau protein by a yeast prion domain *in vitro* as well as in a tau transgenic mouse model.

3.1. *Early experimental/observational data*

3.1.1. *In vitro seeding of Tau by Sup35NM*

To study the potential of the yeast Sup35 prion for tau protein seeding, we expressed the Sup35NM prion domain in *E. coli* and aggregated it at 4 °C into fibrils (Figure S2). Recombinant -5TyrSup35NM [40] was used as a non-aggregated seeding control.

First, we analyzed whether Sup35NM fibrils were capable of directly inducing tau aggregation *in vitro*. The naturally unfolded tau protein does not easily aggregate *in vitro* without co-factors such as heparin. Hence, we incubated either aggregation prone recombinant human 2N4R P301S tau

monomers (Figure 1) or 2N4R wild-type tau monomers (Figure 2) in the presence of very low levels of heparin (0.02 mg/ml) either unseeded (Figure 1a, Figure 2a), with non-aggregated -5TyrSup35NM monomers (Figure 1b, Figure 2b), or with Sup35NM fibril fragments (obtained by sonication of Sup35NM fibrils; Figures 1c and 2c).

Seeding of P301S tau monomers with Sup35NM fibril fragments resulted in the formation of tau filaments displaying a distinctly wavy, slightly skewed corkscrew-like pattern (Figure 1c), which was only rarely seen in unseeded or -5TyrSup35NM incubated tau samples. As this corkscrew-like P301S tau fibril pattern was somewhat reminiscent of the curly tau fibrils previously described upon seedings with mutant tau seeds [41], we hypothesize that it might correspond to a specific tau fibril strain.

As expected, unseeded P301S tau monomers aggregated into a wide variety of filaments (Figure 1a). P301S tau monomers kept with -5TyrSup35NM monomers formed similar filaments as the unseeded sample. However, after 3 days of incubation these filaments showed clumpy protein decorations that were no longer visible after 16 days (Figure 1b). This suggested a transient interaction of the tau monomers with -5TyrSup35NM, which itself also formed fibrils after 16 days when kept without tau monomers (Figure S3).

Strikingly, Sup35NM seeds also provoked rapid aggregation of 2N4R wild-type tau monomers into fibrils (Figure 2c). These fibrils again exhibited a characteristic corkscrew-like pattern, with a clear preference towards regular wave patterns with a periodicity of around 143 nm (± 12 nm, n = 87) (Figure 2c) when observed in negative stain transmission electron microscopy (TEM) images. Compared to non-seeded (Figure 2a) or -5TyrSup35NM exposed wild-type tau monomers (Figure 2b), fibril formation was promoted by Sup35NM seeds, and corkscrew-like fibrils formed already at day 3 (Figure 2c). Immuno-gold labelling of the Sup35NM-induced corkscrew-like fibrils confirmed that they consist of tau, and not of minute amounts of Sup35NM seeds (Figure S4).

Next, we created short seeds out of Sup35NM-induced human wild-type 2N4R tau filaments by sonication to seed 2N4R wild-type tau monomers. After 3 days we observed rapid formation of tau filaments (Figure 3a), which were used for seeding of a next generation of fibrils (Figure 3b). While

the aggregation-accelerating effect of the seeds remained, the distinct wave pattern, which was well preserved in the first generation of re-seeding, lost its exclusivity during the subsequent seeding generation, while morphologies previously seen in heparin-induced samples occurred more often. This observed dilution could be attributable to the fact that Sup35NM-induced fibrils occur first due to the aggregation-promoting effect of Sup35NM. At later time points it is possible that the interaction of tau monomers with heparin is sufficient for fibril formation (as seen for the negative control without Sup35NM seeds in Figure 2a). These heparin-induced tau fibrils could then act as templates, and therefore the morphology of Sup35NM-induced fibrils becomes less dominant.

3.1.2 *In vivo seeding of Tau by Sup35NM*

Next, we asked the question of whether Sup35NM fibrils have the potential to accentuate tau pathology *in vivo.* To this end, we inoculated pre-aggregated Sup35NM fibrils into the hippocampus of P301S mice. These mice express the human full-length 0N4R tau isoform with the P301S tau mutation present in hereditary forms of frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17TAU) [42-44]. P301S tau mice develop slowly progressive intraneuronal tau inclusions, which become accentuated upon the inoculation of β-sheet structured tau seeds [44]. Sup35NM, -5TyrSup35NM and brain homogenate of non-transgenic C57BL/6 mice (termed B6-bh) were used as seeding material for comparison. We inoculated 2.5 μl of Sup35NM pre-formed fibrils, or -5TyrSup35NM, or B6-bh solutions into the right hippocampus of 3-months-old P301S tau transgenic mice. At three months post-inoculation, a strong accentuation of tau pathology was noted at the hippocampal inoculation site and along the injection canal (Figure 4a-d, Figure 5). The accentuation of tau pathology in the CA3 field upon Sup35NM injection was consistent when comparing these mice to B6-bh-injected P301S controls (Figure 4a, c, Table S1). As Sup35NM only remains non-aggregated when high amounts of detergents are present, i.e. conditions incompatible with *in vivo* experiments, we used the less aggregating -5TyrSup35NM mutant as negative control. Indeed, only a mild, non-significant increase in tau pathology was noted in -5TyrSup35NM seeded mice when compared to B6-bh inoculated mice (Figure 4a, c, Table S1). This observation paralleled our *in vitro* findings of a limited short-term interaction between -5TyrSup35NM and P301S tau that

did not obviously change tau fibril structure. Moreover, our experiment also confirmed the strong aggregation propensity of Sup35NM, which partly persists even with the -5Tyr deletion [45].

Quantitative estimates confirmed an increase in hippocampal tau pathology in the CA3 sector of the Sup35NM inoculated ipsilateral hippocampus when compared to the contralateral (non-seeded) side (Figure 4b, d, Table S2). Similar to the seeding effects of brain extracts from tauopathy patients in P301S host mice [46], we noted the induction of Gallyas-Braak positive grains in the dorsal fornix in a subset of Sup35NM seeded mice, mostly in close proximity to the inoculation canal (Figure 5a, b). These findings are compatible with a robust *in vivo* seeding effect of Sup35NM and mild, non-significant residual seeding effects of the less aggregation prone -5TyrSup35NM mutant.

3.1.1 *Future Experiments and Validation Studies*

Our proof of concept experiments demonstrating cross-species cross-seeding of tau protein by a yeast prion should be validated in other model systems, studying the cross-seeding potential of other non-mammalian prions with tau and other neurodegenerative prion-like proteins. In the context of AD, cross-seeding of Sup35 and amyloid-β should be studied. Like tau, amyloid-β is susceptible to cross-seeding, e.g. by IAPP [47, 48] and α-synuclein [17].

Further studies should characterize the interaction of various bacterial and plant prions [20-24] with tau and amyloid-β. As a more complex task, the search for yet unidentified non-mammalian prion proteins, which may act as exogenous seeds or which are generated by the human microbiome, will be instrumental. Recently developed algorithms to identify proteins with prion-like properties may support the discovery of such microbiome-associated prions [20, 49, 50]. To make the case for a truly pathogenetic role of non-mammalian prions in the pathogenesis of AD, clinical studies will eventually be required to investigate the prevalence of such microbiological or exogenous prions e.g. in the nasal cavity or the olfactory bulb of AD patients. Identification of specific non-mammalian prion seeds could ultimately result in novel preventive, diagnostic and therapeutic approaches to neurodegenerative disorders.

4. Major challenges for the hypothesis

Here, we provide first evidence for tau seeding by an archaic yeast prion. While our observations serve well as a proof of principle, it remains unclear whether yeast prions play a relevant role in the pathogenesis of AD or other tauopathies. To this end, analyzing e.g. the presence of microbiome-associated prions in AD patients will be instrumental (see Future Experiments and Validation Studies).

The observed tau seeding effect of Sup35NM (and our hypothesis) suggest that certain (archaic) prions might retain a broad seeding capacity. To verify this assumption, the interaction of various prions and prion-like proteins from different species will have to be studied. This may part prions into a hierarchic cross-seeding system, separating highly cross-seeding competent prions from others, which might be more "seedable". Some recent data would well support such a hierarchic cross-seeding system: e.g., IAPP aggregates were able to seed α-synuclein monomers, but not vice versa [51]; *E. coli*-derived Curli protein aggregates seeded α-synuclein, while they were unable to seed tau monomers [52].

In light of the growing evidence for disease-specific fibril strains in tauopathies, our hypothesis will also have to face the challenge of whether non-mammalian prions will be able to induce tau strains similar to those detected in tauopathy patients [3-5]. Future cryo-EM studies may provide insights into the strain structures of cross-seeded tau aggregates.

Next, we discuss how our hypothesis addresses the critical questions confronting all major theories of Alzheimer's dementia, as suggested by Khachaturian et al. [53].

A non-mammalian prion-induced templating of tau protein in AD would well explain the characteristic association of the disease with age. Templated seedings of neurodegenerative prion-like proteins are typically associated with very long incubation periods, even when seed and seeded protein share their primary structure. Incubation times of up to three decades were noted in patients with iatrogenic PrP^{Sc} infections or amyloid-β transmissions by contaminated growth hormone or gonadotropin [54, 55], reaching even five decades in people infected with Kuru via cannibalism [56]. In this context,

exposure to microbiotic or exogenous non-mammalian prions early in life would be followed by a long incubation period in sporadic AD. Thus, our putative theory might explain the relationship between the biology of aging and the biology of AD pathology.

Genome-wide association studies identified a set of genetic variants in AD which were mapping to distinct biological pathways, including protein misfolding, protein ubiquitination as well as endocytosis [57, 58]. These latter pathways would be in good agreement with our hypothesis of a prion-induced provocation of tau misfolding. All three biological pathways are directly linked to prion-like protein aggregation and would be expected to be involved in heterotypic tau seeding.

In the context of AD risk factors, type 2 diabetes has increasingly been studied [59-61]. Recently, heterotypic seeding of tau by a diabetes-associated prion-like protein has been reported. Diabetes associated IAPP was shown to cross-seed tau as well as α-synuclein [19, 51]. This provides an example of structure-based heterotypic, yet still mammalian protein based cross-seeding of amyloid aggregates in the context of AD and relates well to our proposed induction of tau aggregation by non-mammalian β-structured templates. In this context, our hypothesis may also explain the relationships between known risk or protective factors and the neurobiological mechanisms by which they modify risk.

Our hypothesis assumes, amongst others, a primarily transnasal route of tau cross-seeding by either exogenous non-mammalian prions or prions generated by the microbiome of the nasal cavity. Heterotypic non-mammalian prions may enter the cranium from the nasopharyngeal cavity similar to viruses [62, 63]. From the olfactory bulb they may follow the olfactory nerve, which projects to the entorhinal cortex, the amygdala and the hippocampus [64, 65]. Heterotypic cross-seeding of tau may occur in the first neurons of the olfactory network, as early tau pathology has been described in the olfactory system [66, 67]. Once tau aggregation is initiated, it can subsequently spread by its own via trans-neuronal spreading [6, 68], resulting in the characteristic Braak's stages of tau pathology progression in AD [69]. In line with this, olfactory dysfunction occurs as an early symptom in many patients with mild cognitive impairment (MCI) or AD [70-73]. The proposed pattern of spreading of tau aggregation from the olfactory bulb towards the limbic system, with subsequent involvement of

cortical structures also mirrors the clinical course and progression of tau pathology in AD [74, 75]. As our hypothesis addresses the very first step of tau aggregation in AD, it is in full compliance with all present knowledge on the course of tau pathology and associated neuronal dysfunction in AD. It therefore not only explains how and when the clinical manifestations relate to pathophysiology, but also provides a plausible explanation for the anatomical specificity of early lesions associated with AD.

Our hypothesis also accounts well for the mixed pathology noted in AD and other tauopathies, where different neurodegenerative prion-like proteins co-occur. Tau can be seeded by various other neurodegenerative prion-like proteins, including α-synuclein [14, 15, 76] and amyloid-β [9, 18], as well as by non-neurodegenerative prion-like proteins like IAPP [19]. Furthermore, tau has the ability to form various tau strains which acquire unique conformational features and quaternary structures [3, 4, 77-79]. The transmission of distinct tau strains is specific to unique pathological conformations [80]. Therefore, different non-mammalian prion seeds could provoke the formation of different tau strains, resulting in different disease manifestations and clinical heterogeneity.

Our theory is also in line with the importance of tau as a biomarker, both in the cerebrospinal fluid as well as in AD imaging. With our hypothesis currently still at a proof of concept stage, detailed considerations on future biomarkers targeting non-mammalian prions for prognosis and risk stratification in AD would be premature. In the case of identification, non-mammalian prions related to AD might be targeted with preventive, diagnostic and/or therapeutic intentions in the future.

4. Linkage to other major theories

Here, we propose that seeding of tau and/or amyloid-β by non-mammalian prion proteins might constitute a key trigger event in the cascade of AD. The amyloid cascade hypothesis or amyloid-β hypothesis, established in the 1990ies, postulated a crucial role of amyloid-β aggregation for the pathogenesis of AD, considering tau as a downstream effector [81, 82]. Our hypothesis is compatible with this idea of tau aggregation as a downstream event in the cascade of AD, but modifies the amyloid hypothesis towards a model where cross-seeding of tau and amyloid-β can also be provoked by heterotypic non-mammalian prions.

Our present hypothesis is related to the rapidly growing number of discoveries of non-mammalian prions in fungi, bacteria, plants and even viruses [7, 20-25]. This recent diversification of the prion family was paralleled by the notion that several prions share similar functions. As an example, tau and Sup35 are both involved in phase separation [34, 35], and a prebiotic regulatory function of amyloids has been suggested [83]. This opens up the perspective that prions may interact across species, even though species barriers have previously been established for many prion disorders [84]. Our hypothesis thus extends the prion hypothesis to prion cross-infections in the context of neurodegenerative disorders.

An infectious pathway has been proposed earlier for AD, outlining a potential route of infection reaching the limbic system from the nasal cavity via olfactory bulb and nerve [66, 85]. The viral infectious hypothesis builds on the predominant limbic involvement in cerebral HSV infections and the early affection of the limbic system in AD [86], and the occurrence of neurofibrillary tangles as possible late sequelae of early infections with measles [87]. However, in most infection related models, the direct mechanistic link from the proposed viral or bacterial infection to the characteristic protein misfolding of tau or amyloid-β remains yet unidentified [88]. Our hypothesis provides a structural basis for a prion-induced misfolding of tau or amyloid-β into prion-like β-structured fibrils upon a protein-only infection by non-mammalian prion particles.

In analogy to our proposed model of a transnasal infectious pathway in AD, an initial induction of α-synuclein aggregation by a yet unknown agent in the gut has been proposed by Braak and coworkers

for Parkinson's disease [89]. Recently, a gut bacterial prion was found to cross-seed α-synuclein [52]. This supports our present hypothesis of a non-mammalian prion-mediated induction of tau and amyloid-β aggregation in AD and other tauopathies.

As AD and other tauopathies present with clinical and pathological heterogeneity [90, 91], even within familial forms of AD [92], we are well aware that various pathomechanisms may co-occur and interplay in these disorders. Amongst others, the induction of tau aggregation is known to be facilitated by other structural changes, e.g. tau mutations, which render the soluble tau protein more aggregation prone [93, 94]. Tau cleavage may furthermore generate tau fragments which can seed full-length tau into toxic aggregates [95]. Our present hypothesis shall therefore not be misunderstood as an exclusive proposition, but rather as an extension to the spectrum of possible pathogenetic factors provoking AD and other tauopathies.

Acknowledgments

We thank Prof. Reed Wickner, Laboratory of Biochemistry and Genetics, NIH, Bethesda, U.S.A., for his advice and for providing the Sup35NM plasmid, and Dr. Michel Goedert, MRC, Laboratory of Molecular Biology, Cambridge CB2, UK for his advice and for providing the P301S tau transgenic mice.

Funding

This work was in part supported by the Swiss National Science Foundation (31003A_152846 to MT, 32323B_123812 and 310030_169486 to DTW, 310031_188548 and CRSII5_177195 to HS), the Mach-Gaensslen Foundation, and the Synapsis Foundation Switzerland (to MT, DTW, and HS).

Author Contributions

DTW conceived and designed the study; MF, CL, HS and DTW designed research; MF performed the Sup35NM expression and purification, MF and ZS performed the *in vivo* experiments and light microscopy, CL performed the *in vitro* experiments and electron microscopy; MF, CL, AM, HS, and DTW analyzed data; DTW drafted the manuscript. All authors discussed the results and commented on the manuscript. All authors read and approved the final manuscript.

Supplementary Data

Supplementary data - Material and Methods

Supplementary data - Figures and Tables

Declaration of Competing Interest

The authors declare no competing interests

References

[1] Goedert M, Eisenberg DS, Crowther RA. Propagation of Tau Aggregates and Neurodegeneration. Annu Rev Neurosci. 2017;40:189-210.
[2] Rochet JC, Lansbury PT, Jr. Amyloid fibrillogenesis: themes and variations. Curr Opin Struct Biol. 2000;10:60-8.
[3] Fitzpatrick AWP, Falcon B, He S, Murzin AG, Murshudov G, Garringer HJ, et al. Cryo-EM structures of tau filaments from Alzheimer's disease. Nature. 2017;547:185-90.
[4] Falcon B, Zivanov J, Zhang W, Murzin AG, Garringer HJ, Vidal R, et al. Novel tau filament fold in chronic traumatic encephalopathy encloses hydrophobic molecules. Nature. 2019;568:420-3.
[5] Falcon B, Zhang W, Murzin AG, Murshudov G, Garringer HJ, Vidal R, et al. Structures of filaments from Pick's disease reveal a novel tau protein fold. Nature. 2018;561:137-40.
[6] Clavaguera F, Bolmont T, Crowther RA, Abramowski D, Frank S, Probst A, et al. Transmission and spreading of tauopathy in transgenic mouse brain. Nat Cell Biol. 2009;11:909-13.
[7] Prusiner SB. Biology and genetics of prions causing neurodegeneration. Annu Rev Genet. 2013;47:601-23.
[8] Goedert M. NEURODEGENERATION. Alzheimer's and Parkinson's diseases: The prion concept in relation to assembled Abeta, tau, and alpha-synuclein. Science. 2015;349:1255555.
[9] Vasconcelos B, Stancu IC, Buist A, Bird M, Wang P, Vanoosthuyse A, et al. Heterotypic seeding of Tau fibrillization by pre-aggregated Abeta provides potent seeds for prion-like seeding and propagation of Tau-pathology in vivo. Acta neuropathologica. 2016;131:549-69.
[10] Chaudhuri P, Prajapati KP, Anand BG, Dubey K, Kar K. Amyloid cross-seeding raises new dimensions to understanding of amyloidogenesis mechanism. Ageing Res Rev. 2019;56:100937.
[11] Nelson R, Sawaya MR, Balbirnie M, Madsen AO, Riekel C, Grothe R, et al. Structure of the cross-beta spine of amyloid-like fibrils. Nature. 2005;435:773-8.
[12] Jarrett JT, Lansbury PT, Jr. Seeding "one-dimensional crystallization" of amyloid: a pathogenic mechanism in Alzheimer's disease and scrapie? Cell. 1993;73:1055-8.
[13] Guo T, Noble W, Hanger DP. Roles of tau protein in health and disease. Acta neuropathologica. 2017;133:665-704.
[14] Giasson BI, Forman MS, Higuchi M, Golbe LI, Graves CL, Kotzbauer PT, et al. Initiation and synergistic fibrillization of tau and alpha-synuclein. Science. 2003;300:636-40.
[15] Guo JL, Covell DJ, Daniels JP, Iba M, Stieber A, Zhang B, et al. Distinct alpha-synuclein strains differentially promote tau inclusions in neurons. Cell. 2013;154:103-17.
[16] Castillo-Carranza DL, Guerrero-Munoz MJ, Sengupta U, Gerson JE, Kayed R. alpha-Synuclein Oligomers Induce a Unique Toxic Tau Strain. Biol Psychiatry. 2018;84:499-508.
[17] Clinton LK, Blurton-Jones M, Myczek K, Trojanowski JQ, LaFerla FM. Synergistic Interactions between Abeta, tau, and alpha-synuclein: acceleration of neuropathology and cognitive decline. J Neurosci. 2010;30:7281-9.
[18] Guo JP, Arai T, Miklossy J, McGeer PL. Abeta and tau form soluble complexes that may promote self aggregation of both into the insoluble forms observed in Alzheimer's disease. Proc Natl Acad Sci U S A. 2006;103:1953-8.
[19] Arya S, Claud SL, Cantrell KL, Bowers MT. Catalytic Prion-Like Cross-Talk between a Key Alzheimer's Disease Tau-Fragment R3 and the Type 2 Diabetes Peptide IAPP. ACS Chem Neurosci. 2019;10:4757-65.
[20] Yuan AH, Hochschild A. A bacterial global regulator forms a prion. Science. 2017;355:198-201.
[21] Chapman MR, Robinson LS, Pinkner JS, Roth R, Heuser J, Hammar M, et al. Role of Escherichia coli curli operons in directing amyloid fiber formation. Science. 2002;295:851-5.
[22] Shahnawaz M, Park KW, Mukherjee A, Diaz-Espinoza R, Soto C. Prion-like characteristics of the bacterial protein Microcin E492. Scientific reports. 2017;7:45720.
[23] Chakrabortee S, Kayatekin C, Newby GA, Mendillo ML, Lancaster A, Lindquist S. Luminidependens (LD) is an Arabidopsis protein with prion behavior. Proc Natl Acad Sci U S A. 2016;113:6065-70.
[24] Antonets KS, Nizhnikov AA. Amyloids and prions in plants: Facts and perspectives. Prion. 2017;11:300-12.

[25] Nan H, Chen H, Tuite MF, Xu X. A viral expression factor behaves as a prion. Nat Commun. 2019;10:359.
[26] Gorkovskiy A, Thurber KR, Tycko R, Wickner RB. Locating folds of the in-register parallel beta-sheet of the Sup35p prion domain infectious amyloid. Proc Natl Acad Sci U S A. 2014;111:E4615-22.
[27] Derkatch IL, Bradley ME, Hong JY, Liebman SW. Prions affect the appearance of other prions: the story of [PIN(+)]. Cell. 2001;106:171-82.
[28] Du Z, Park KW, Yu H, Fan Q, Li L. Newly identified prion linked to the chromatin-remodeling factor Swi1 in Saccharomyces cerevisiae. Nat Genet. 2008;40:460-5.
[29] Lundmark K, Westermark GT, Olsen A, Westermark P. Protein fibrils in nature can enhance amyloid protein A amyloidosis in mice: Cross-seeding as a disease mechanism. Proc Natl Acad Sci U S A. 2005;102:6098-102.
[30] Halfmann R, Jarosz DF, Jones SK, Chang A, Lancaster AK, Lindquist S. Prions are a common mechanism for phenotypic inheritance in wild yeasts. Nature. 2012;482:363-8.
[31] Kelly AC, Busby B, Wickner RB. Effect of domestication on the spread of the [PIN+] prion in Saccharomyces cerevisiae. Genetics. 2014;197:1007-24.
[32] Wickner RB. [URE3] as an altered URE2 protein: evidence for a prion analog in Saccharomyces cerevisiae. Science. 1994;264:566-9.
[33] Sparrer HE, Santoso A, Szoka FC, Jr., Weissman JS. Evidence for the prion hypothesis: induction of the yeast [PSI+] factor by in vitro- converted Sup35 protein. Science. 2000;289:595-9.
[34] Koren SA, Hamm MJ, Meier SE, Weiss BE, Nation GK, Chishti EA, et al. Tau drives translational selectivity by interacting with ribosomal proteins. Acta neuropathologica. 2019;137:571-83.
[35] Franzmann TM, Jahnel M, Pozniakovsky A, Mahamid J, Holehouse AS, Nuske E, et al. Phase separation of a yeast prion protein promotes cellular fitness. Science. 2018;359.
[36] Ohhashi Y, Ito K, Toyama BH, Weissman JS, Tanaka M. Differences in prion strain conformations result from non-native interactions in a nucleus. Nat Chem Biol. 2010;6:225-30.
[37] Krammer C, Kryndushkin D, Suhre MH, Kremmer E, Hofmann A, Pfeifer A, et al. The yeast Sup35NM domain propagates as a prion in mammalian cells. Proc Natl Acad Sci U S A. 2009;106:462-7.
[38] Glover JR, Kowal AS, Schirmer EC, Patino MM, Liu JJ, Lindquist S. Self-seeded fibers formed by Sup35, the protein determinant of [PSI+], a heritable prion-like factor of S. cerevisiae. Cell. 1997;89:811-9.
[39] Chen CY, Rojanatavorn K, Clark AC, Shih JC. Characterization and enzymatic degradation of Sup35NM, a yeast prion-like protein. Protein Sci. 2005;14:2228-35.
[40] Gonzalez Nelson AC, Paul KR, Petri M, Flores N, Rogge RA, Cascarina SM, et al. Increasing prion propensity by hydrophobic insertion. PLoS One. 2014;9:e89286.
[41] Frost B, Ollesch J, Wille H, Diamond MI. Conformational diversity of wild-type Tau fibrils specified by templated conformation change. J Biol Chem. 2009;284:3546-51.
[42] Yoshiyama Y, Higuchi M, Zhang B, Huang SM, Iwata N, Saido TC, et al. Synapse loss and microglial activation precede tangles in a P301S tauopathy mouse model. Neuron. 2007;53:337-51.
[43] Holmes BB, Furman JL, Mahan TE, Yamasaki TR, Mirbaha H, Eades WC, et al. Proteopathic tau seeding predicts tauopathy in vivo. Proc Natl Acad Sci U S A. 2014;111:E4376-85.
[44] Ahmed Z, Cooper J, Murray TK, Garn K, McNaughton E, Clarke H, et al. A novel in vivo model of tau propagation with rapid and progressive neurofibrillary tangle pathology: the pattern of spread is determined by connectivity, not proximity. Acta neuropathologica. 2014;127:667-83.
[45] Ross ED, Edskes HK, Terry MJ, Wickner RB. Primary sequence independence for prion formation. Proc Natl Acad Sci U S A. 2005;102:12825-30.
[46] Boluda S, Iba M, Zhang B, Raible KM, Lee VM, Trojanowski JQ. Differential induction and spread of tau pathology in young PS19 tau transgenic mice following intracerebral injections of pathological tau from Alzheimer's disease or corticobasal degeneration brains. Acta neuropathologica. 2015;129:221-37.
[47] Roder C, Kupreichyk T, Gremer L, Schafer LU, Pothula KR, Ravelli RBG, et al. Cryo-EM structure of islet amyloid polypeptide fibrils reveals similarities with amyloid-beta fibrils. Nat Struct Mol Biol. 2020.
[48] Moreno-Gonzalez I, Edwards Iii G, Salvadores N, Shahnawaz M, Diaz-Espinoza R, Soto C. Molecular interaction between type 2 diabetes and Alzheimer's disease through cross-seeding of protein misfolding. Mol Psychiatry. 2017;22:1327-34.

[49] Cascarina SM, Ross ED. Natural and pathogenic protein sequence variation affecting prion-like domains within and across human proteomes. BMC Genomics. 2020;21:23.
[50] Alberti S, Halfmann R, King O, Kapila A, Lindquist S. A systematic survey identifies prions and illuminates sequence features of prionogenic proteins. Cell. 2009;137:146-58.
[51] Horvath I, Wittung-Stafshede P. Cross-talk between amyloidogenic proteins in type-2 diabetes and Parkinson's disease. Proc Natl Acad Sci U S A. 2016;113:12473-7.
[52] Sampson TR, Challis C, Jain N, Moiseyenko A, Ladinsky MS, Shastri GG, et al. A gut bacterial amyloid promotes alpha-synuclein aggregation and motor impairment in mice. Elife. 2020;9.
[53] Khachaturian AS, Hayden KM, Mielke MM, Tang Y, Lutz MW, Gold M, et al. New thinking about thinking, part two. Theoretical articles for Alzheimer's & Dementia. Alzheimers Dement. 2018;14:703-6.
[54] Jaunmuktane Z, Mead S, Ellis M, Wadsworth JD, Nicoll AJ, Kenny J, et al. Evidence for human transmission of amyloid-beta pathology and cerebral amyloid angiopathy. Nature. 2015;525:247-50.
[55] Purro SA, Farrow MA, Linehan J, Nazari T, Thomas DX, Chen Z, et al. Transmission of amyloid-beta protein pathology from cadaveric pituitary growth hormone. Nature. 2018;564:415-9.
[56] Collinge J, Whitfield J, McKintosh E, Beck J, Mead S, Thomas DJ, et al. Kuru in the 21st century--an acquired human prion disease with very long incubation periods. Lancet. 2006;367:2068-74.
[57] International Genomics of Alzheimer's Disease C. Convergent genetic and expression data implicate immunity in Alzheimer's disease. Alzheimers Dement. 2015;11:658-71.
[58] Ahmad S, Bannister C, van der Lee SJ, Vojinovic D, Adams HHH, Ramirez A, et al. Disentangling the biological pathways involved in early features of Alzheimer's disease in the Rotterdam Study. Alzheimers Dement. 2018;14:848-57.
[59] Abner EL, Nelson PT, Kryscio RJ, Schmitt FA, Fardo DW, Woltjer RL, et al. Diabetes is associated with cerebrovascular but not Alzheimer's disease neuropathology. Alzheimers Dement. 2016;12:882-9.
[60] Biessels GJ, Nobili F, Teunissen CE, Simo R, Scheltens P. Understanding multifactorial brain changes in type 2 diabetes: a biomarker perspective. Lancet Neurol. 2020.
[61] Bellou V, Belbasis L, Tzoulaki I, Middleton LT, Ioannidis JPA, Evangelou E. Systematic evaluation of the associations between environmental risk factors and dementia: An umbrella review of systematic reviews and meta-analyses. Alzheimers Dement. 2017;13:406-18.
[62] Cairns DM, Rouleau N, Parker RN, Walsh KG, Gehrke L, Kaplan DL. A 3D human brain-like tissue model of herpes-induced Alzheimer's disease. Sci Adv. 2020;6:eaay8828.
[63] Doty RL. The olfactory vector hypothesis of neurodegenerative disease: is it viable? Ann Neurol. 2008;63:7-15.
[64] Nelson PT, Abner EL, Patel E, Anderson S, Wilcock DM, Kryscio RJ, et al. The Amygdala as a Locus of Pathologic Misfolding in Neurodegenerative Diseases. J Neuropathol Exp Neurol. 2018;77:2-20.
[65] Murphy C. Olfactory and other sensory impairments in Alzheimer disease. Nature reviews Neurology. 2019;15:11-24.
[66] Ulrich J. Alzheimer changes in nondemented patients younger than sixty-five: possible early stages of Alzheimer's disease and senile dementia of Alzheimer type. Ann Neurol. 1985;17:273-7.
[67] Tsuboi Y, Wszolek ZK, Graff-Radford NR, Cookson N, Dickson DW. Tau pathology in the olfactory bulb correlates with Braak stage, Lewy body pathology and apolipoprotein epsilon4. Neuropathol Appl Neurobiol. 2003;29:503-10.
[68] Clavaguera F, Akatsu H, Fraser G, Crowther RA, Frank S, Hench J, et al. Brain homogenates from human tauopathies induce tau inclusions in mouse brain. Proc Natl Acad Sci U S A. 2013;110:9535-40.
[69] Braak H, Del Tredici K. The pathological process underlying Alzheimer's disease in individuals under thirty. Acta neuropathologica. 2011;121:171-81.
[70] Lafaille-Magnan ME, Poirier J, Etienne P, Tremblay-Mercier J, Frenette J, Rosa-Neto P, et al. Odor identification as a biomarker of preclinical AD in older adults at risk. Neurology. 2017;89:327-35.
[71] Arnold SE, Lee EB, Moberg PJ, Stutzbach L, Kazi H, Han LY, et al. Olfactory epithelium amyloid-beta and paired helical filament-tau pathology in Alzheimer disease. Ann Neurol. 2010;67:462-9.
[72] Risacher SL, Tallman EF, West JD, Yoder KK, Hutchins GD, Fletcher JW, et al. Olfactory identification in subjective cognitive decline and mild cognitive impairment: Association with tau but not amyloid positron emission tomography. Alzheimers Dement (Amst). 2017;9:57-66.

[73] Palta P, Chen H, Deal JA, Sharrett AR, Gross A, Knopman D, et al. Olfactory function and neurocognitive outcomes in old age: The Atherosclerosis Risk in Communities Neurocognitive Study. Alzheimers Dement. 2018;14:1015-21.
[74] Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol (Berl). 1991;82:239-59.
[75] Braak H, Del Tredici K. Potential Pathways of Abnormal Tau and alpha-Synuclein Dissemination in Sporadic Alzheimer's and Parkinson's Diseases. Cold Spring Harb Perspect Biol. 2016;8.
[76] Moussaud S, Jones DR, Moussaud-Lamodiere EL, Delenclos M, Ross OA, McLean PJ. Alpha-synuclein and tau: teammates in neurodegeneration? Mol Neurodegener. 2014;9:43.
[77] Falcon B, Zhang W, Schweighauser M, Murzin AG, Vidal R, Garringer HJ, et al. Tau filaments from multiple cases of sporadic and inherited Alzheimer's disease adopt a common fold. Acta neuropathologica. 2018;136:699-708.
[78] Kaufman SK, Sanders DW, Thomas TL, Ruchinskas AJ, Vaquer-Alicea J, Sharma AM, et al. Tau Prion Strains Dictate Patterns of Cell Pathology, Progression Rate, and Regional Vulnerability In Vivo. Neuron. 2016;92:796-812.
[79] Collinge J. Mammalian prions and their wider relevance in neurodegenerative diseases. Nature. 2016;539:217-26.
[80] He Z, McBride JD, Xu H, Changolkar L, Kim SJ, Zhang B, et al. Transmission of tauopathy strains is independent of their isoform composition. Nat Commun. 2020;11:7.
[81] Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. Science. 1992;256:184-5.
[82] Selkoe DJ. The molecular pathology of Alzheimer's disease. Neuron. 1991;6:487-98.
[83] Rout SK, Friedmann MP, Riek R, Greenwald J. A prebiotic template-directed peptide synthesis based on amyloids. Nat Commun. 2018;9:234.
[84] Tessier PM, Lindquist S. Unraveling infectious structures, strain variants and species barriers for the yeast prion [PSI+]. Nat Struct Mol Biol. 2009;16:598-605.
[85] Ball MJ, Lukiw WJ, Kammerman EM, Hill JM. Intracerebral propagation of Alzheimer's disease: strengthening evidence of a herpes simplex virus etiology. Alzheimers Dement. 2013;9:169-75.
[86] Itzhaki RF, Lathe R, Balin BJ, Ball MJ, Bearer EL, Braak H, et al. Microbes and Alzheimer's Disease. J Alzheimers Dis. 2016;51:979-84.
[87] Wisniewski K, Jervis GA, Moretz RC, Wisniewski HM. Alzheimer neurofibrillary tangles in diseases other than senile and presenile dementia. Ann Neurol. 1979;5:288-94.
[88] Finch CE, Kulminski AM. The Alzheimer's Disease Exposome. Alzheimers Dement. 2019;15:1123-32.
[89] Braak H, Rub U, Gai WP, Del Tredici K. Idiopathic Parkinson's disease: possible routes by which vulnerable neuronal types may be subject to neuroinvasion by an unknown pathogen. J Neural Transm (Vienna). 2003;110:517-36.
[90] Dujardin S, Commins C, Lathuiliere A, Beerepoot P, Fernandes AR, Kamath TV, et al. Tau molecular diversity contributes to clinical heterogeneity in Alzheimer's disease. Nature medicine. 2020.
[91] Scheltens NME, Tijms BM, Koene T, Barkhof F, Teunissen CE, Wolfsgruber S, et al. Cognitive subtypes of probable Alzheimer's disease robustly identified in four cohorts. Alzheimers Dement. 2017;13:1226-36.
[92] Van der Flier WM. Clinical heterogeneity in familial Alzheimer's disease. Lancet Neurol. 2016;15:1296-8.
[93] Goedert M, Jakes R, Crowther RA. Effects of frontotemporal dementia FTDP-17 mutations on heparin-induced assembly of tau filaments. FEBS Lett. 1999;450:306-11.
[94] Chang E, Kim S, Yin H, Nagaraja HN, Kuret J. Pathogenic missense MAPT mutations differentially modulate tau aggregation propensity at nucleation and extension steps. J Neurochem. 2008;107:1113-23.
[95] Ozcelik S, Sprenger F, Skachokova Z, Fraser G, Abramowski D, Clavaguera F, et al. Co-expression of truncated and full-length tau induces severe neurotoxicity. Mol Psychiatry. 2016.

Figures

Figure 1

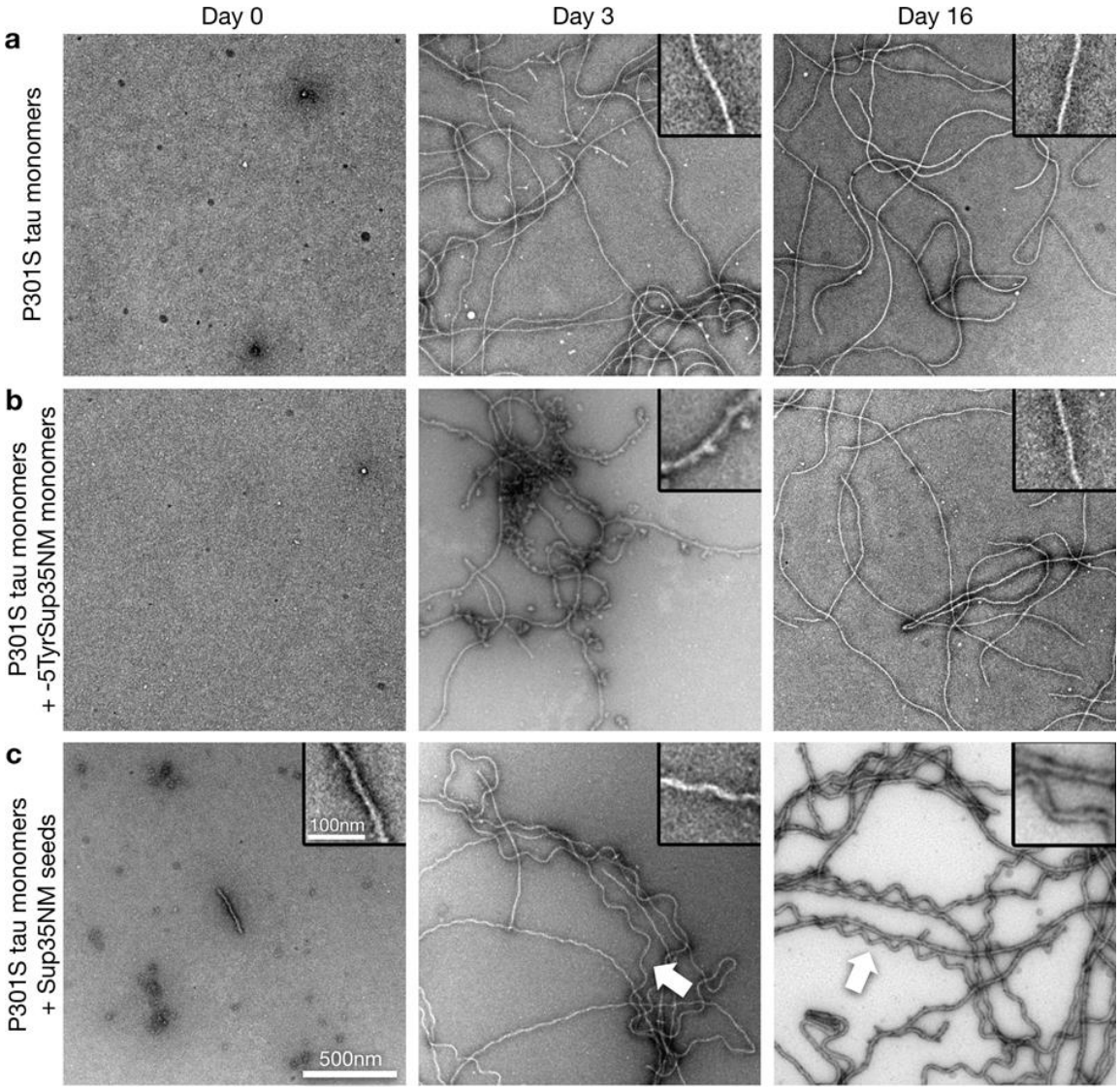


Figure 1. Sup35NM seeds P301S mutant tau aggregation *in vitro*.

a-c, TEM images of negatively stained preparations of human 2N4R P301S mutant tau monomer aggregates, formed under low heparin conditions (a), and after addition of -5TyrSup35NM monomers (b), or Sup35NM seeds (c), at days 0, 3, and 16. Note the occurrence of corkscrew-like shaped tau fibrils at day 3 and 16 upon seeding with Sup35NM fibrils (arrows, c). Scale bar for all images: 500 nm, for all magnified insets: 100 nm.

Figure 2

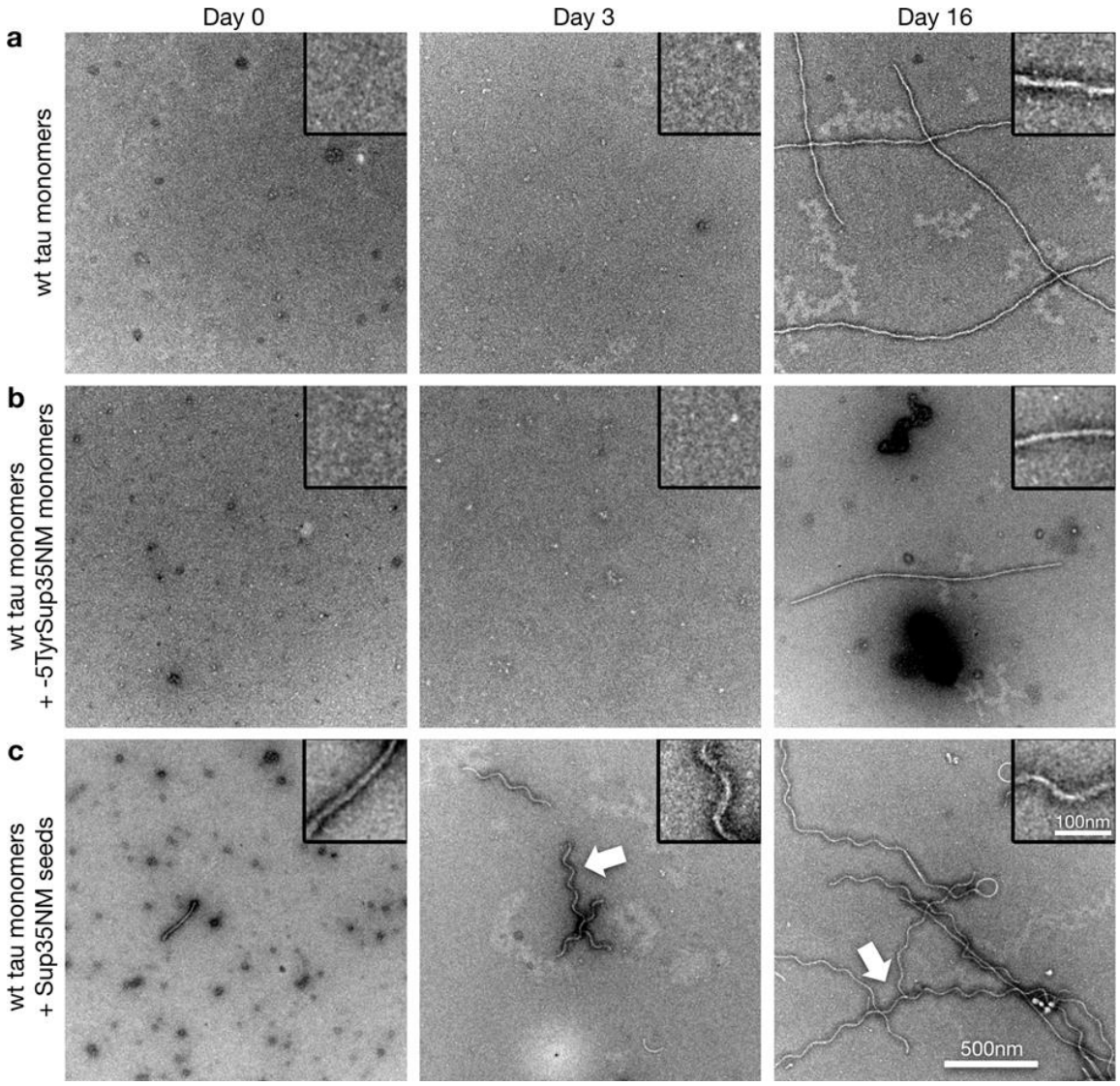


Figure 2. Sup35NM seeds wild-type tau aggregation *in vitro*.

a-c, TEM images of negatively stained preparations of human 2N4R wild-type tau monomer aggregation under low heparin conditions (a), and after addition of -5TyrSup35NM monomers (b), or Sup35NM seeds (c), at days 0, 3, and 16. Note the occurrence of corkscrew-like shaped tau fibrils at day 3 and 16 upon seeding with Sup35NM fibrils (arrows, c). Scale bar for all images: 500 nm, for all magnified insets: 100 nm

Figure 3

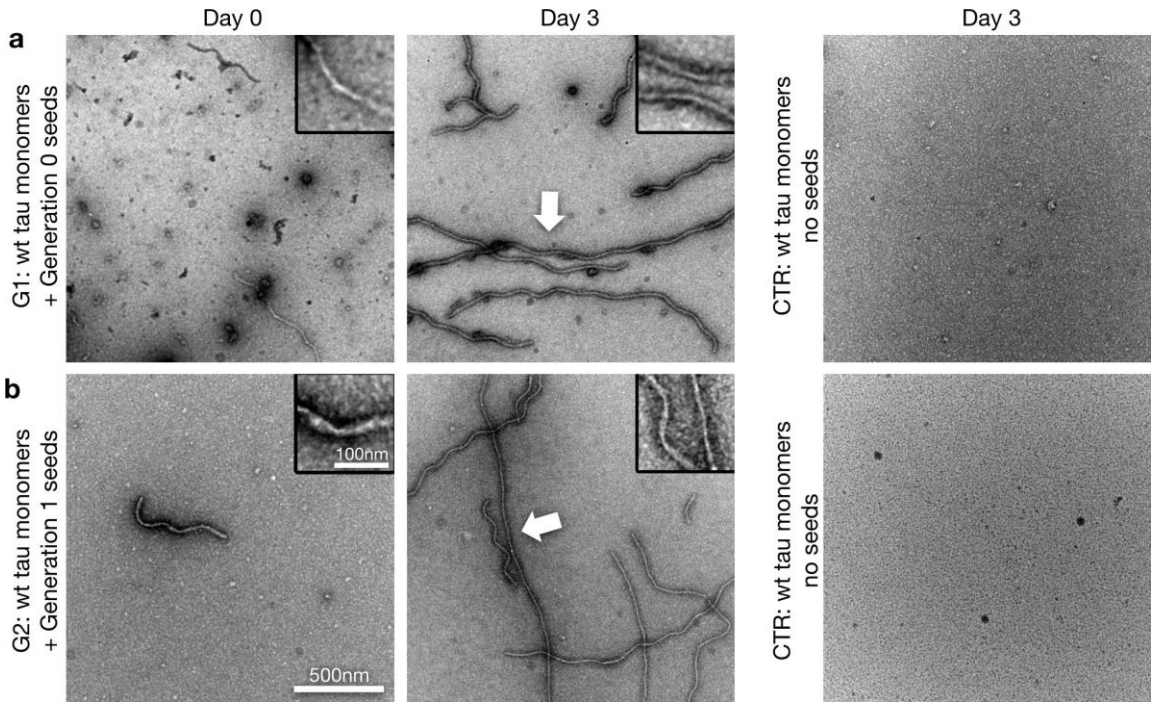


Figure 3. Re-seedings of tau monomers by Sup35NM-induced wild-type tau filaments.

a, b, TEM images of negatively stained preparations of human 2N4R wild-type tau monomer aggregation under low heparin conditions at time points day 0 and day 3, using corkscrew-like 2N4R wild-type tau filaments (termed Generation 0 seeds), which were previously induced by Sup35NM seeds. This resulted in a next generation (Generation 1 (G1)) of corkscrew-like filaments (arrow, a). Re-seeding with G1 filaments again induced corkscrew-like tau aggregates in generation 2 (G2) (arrow, b). Scale bar for all images: 500 nm, for all magnified insets: 100 nm.

Figure 4

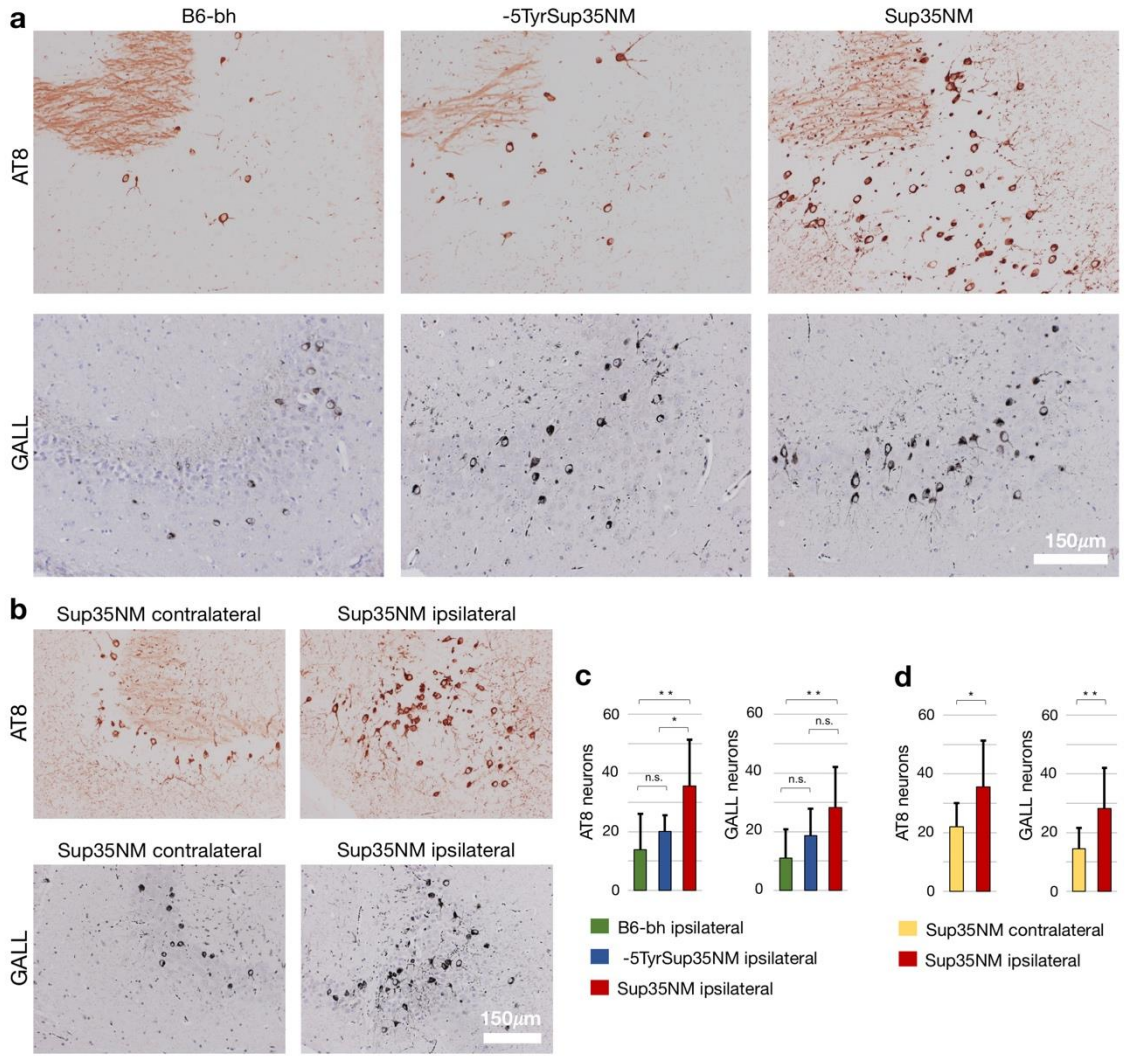


Figure 4. Sup35NM seeds tau aggregation in P301S tau transgenic mice.

a-d, increase in AT8 immunohistochemistry (AT8) and Gallyas-Braak staining (GALL) positive neurons in the unilateral CA3 field after intrahippocampal Sup35NM fibril inoculation in P301S tau transgenic mice. AT8 and Gallyas-Braak positive neurons comparing P301S tau mice inoculated with C57BL/6 mouse brain homogenates (B6-bh), -5TyrSup35NM solution, and Sup35NM fibrils (a, c; one-way ANOVA, followed by Tukey's test), and corresponding histology (Bregma level -2.6mm). Comparison of the non-injected (contralateral) to the injected (ipsilateral) side (b, d; paired T-tests), and representative histological findings (Bregma level -2.4mm). *p<0.05, **p<0.01, n.s. = non-significant; indicated is the mean positive neuron number in CA3 per section ± standard deviation. For detailed quantitative data see Table S2.

Figure 5

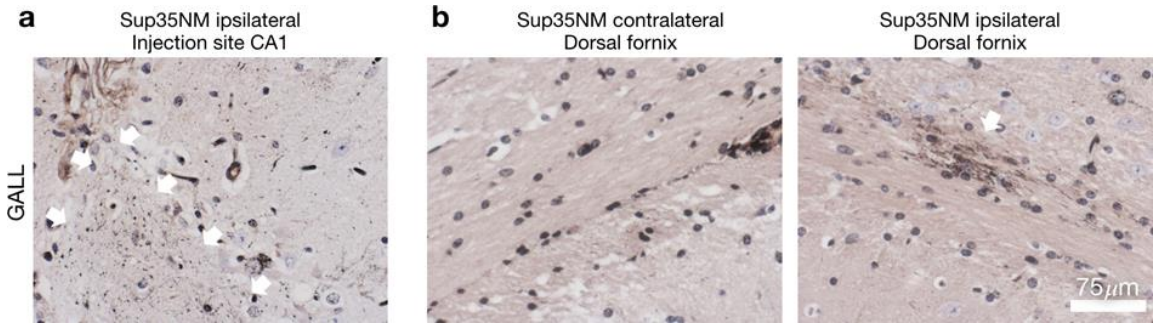


Figure 5. Sup35NM induces focal tau pathology

Focal tau pathology induced around the hippocampal inoculation canal and the ipsilateral dorsal fornix upon Sup35NM inoculation. a, b, Gallyas-Braak stains. Note the characteristic focal grain-like tau aggregates in proximity of the hippocampal injection canal upon Sup35NM inoculation (a, arrows indicate tau pathology along the inoculation canal). Granular tau pathology develops in the ipsilateral dorsal fornix of a SUP35NM-seeded P301S mouse (b, right; arrow), but is absent in the contralateral fornix (b, left).

2.1.1 Supplementary data - Figures and Tables

1. Supplementary Figures

Figure S1

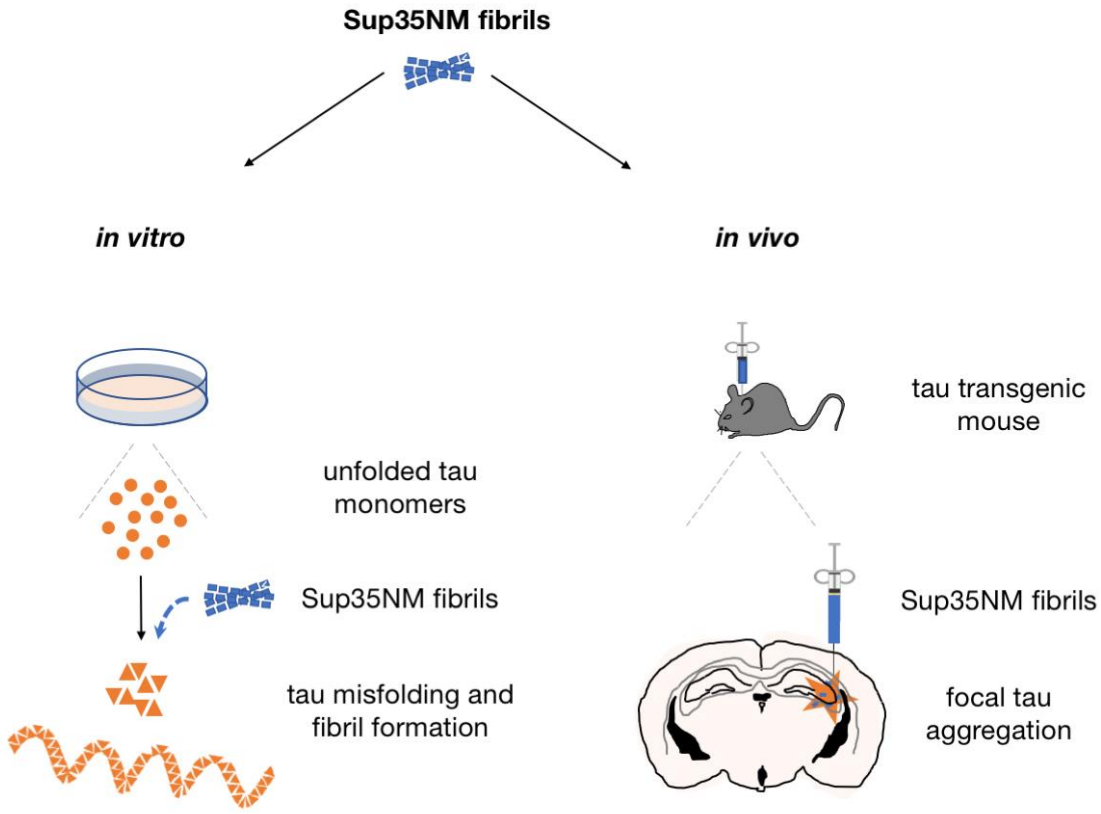


Figure S1. Schematic of our proof of concept experiments.

Tau aggregation is a defining characteristic of a number of human neurodegenerative diseases, including Alzheimer's disease. The cause of tau aggregation in neurodegenerative disorders has yet remained unclear. We hypothesize that seeding of tau protein by non-mammalian prions my play a role in the pathogenesis of tauopathies.

Sup35 is a prion protein which forms heritable aggregates in yeast. Sup35 aggregates show similar β-sheet structures as tau fibrils. As a proof of concept, we here study whether the Sup35NM prion domain can promote the aggregation of human tau. *In vitro*, Sup35NM fibrils provoke accelerated tau misfolding, resulting in increased formation of corkscrew-shaped tau fibrils. *In vivo*, we find that

inoculation of tau transgenic mice with fibrils of the Sup35NM prion domain accentuates tau pathology in the hippocampus of recipient animals.

This demonstrates that an archaic non-human prion can seed the aggregation of a human prion-like protein in a heterotypic manner.

Figure S2

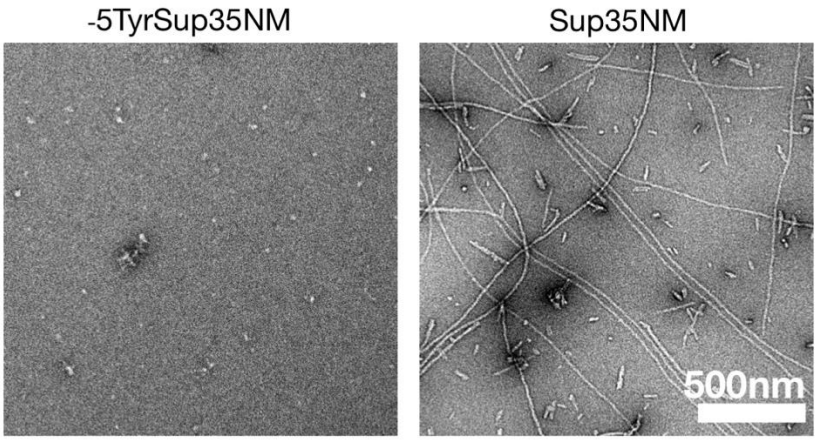


Figure S2. -5TyrSup35NM and Sup35NM preparations

TEM images of negatively stained -5TyrSup35NM and Sup35NM preparations used for intrahippocampal inoculations.

Figure S3

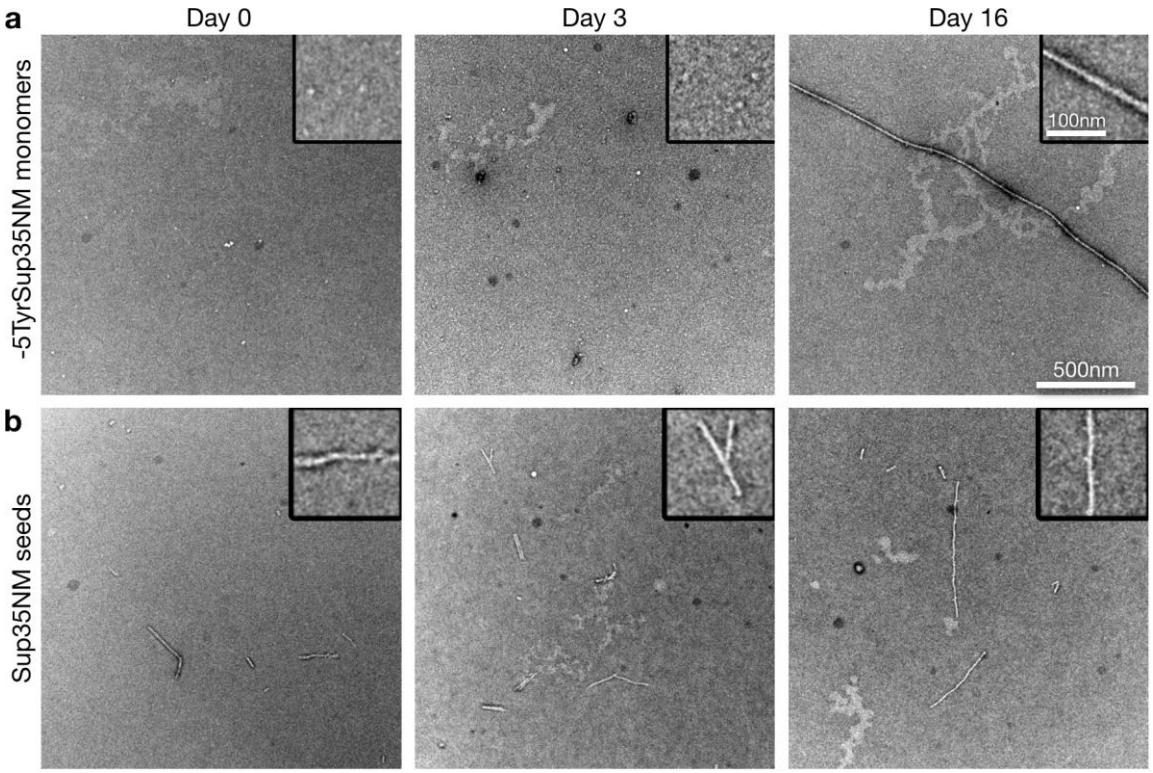


Figure S3. *In vitro* seeding controls.

a, b, TEM images of negatively stained -5TyrSup35NM monomer (a), or Sup35NM seed (b) aggregation under low heparin conditions at days 0, 3, and 16 in the absence of tau monomers. Scale bar for all images: 500 nm, for all magnified insets: 100 nm.

Figure S4

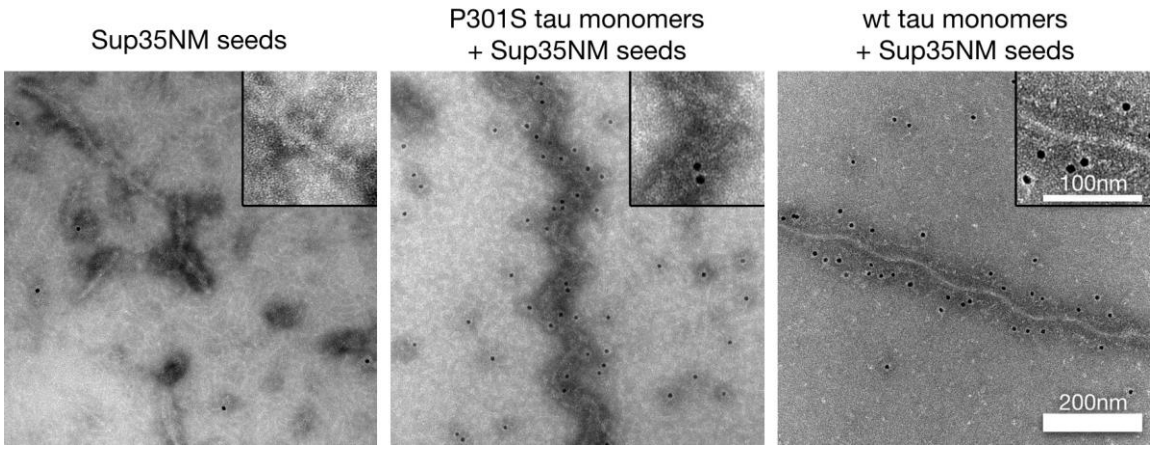


Figure S4. Immuno-gold labelling of Sup35NM-induced P301S mutant tau filaments and Sup35NM-induced wild-type tau filaments

Immuno-gold labelling with the HT7 antibody is negative for Sup35NM seeds alone, but labels the corkscrew-like fibrils obtained upon seeding of P301S tau monomers or wild-type tau monomers with Sup35NM seeds, confirming that these filaments consist of tau. Scale bar for all images: 200 nm, for all magnified insets: 100 nm.

2. Supplementary Tables

Table S1

Quantitative data of AT8 immunohistochemistry and Gallyas-Braak silver stain positive neurons comparing tau pathology between B6-bh injected mice, -5TyrSup35NM inoculated mice, and Sup35NM seeded mice (mean ± SD).

	B6-bh	**-5TyrSup35NM**	**Sup35NM**	**ANOVA, p-Values (adjusted for multiple testing)**
Mean AT8 positive neurons in CA3 ± SD	13.95 ± 12.14 (n=10)	29.18 ± 5.43 (n=8)	35.55 ± 15.70 (n=7)	ANOVA: p= 0.004 p= 0.511 (B6-bh versus -5TyrSup35NM) p= 0.047 (-5TyrSup35NM versus Sup35NM) p= 0.003 (B6-bh versus Sup35NM)
Mean Gallyas-Braak positive neurons in CA3 ± SD	11.03 ± 9.75 (n=10)	18.74 ± 9.10 (n=9)	28.18 ± 13.92 (n=9)	ANOVA: p= 0.009 p= 0.301 (B6-bh versus -5TyrSup35NM) p= 0.188 (-5TyrSup35NM versus Sup35NM) p= 0.007 (B6-bh versus Sup35NM)

Table S2

Quantitative data of AT8 immunohistochemistry and Gallyas-Braak silver stain positive neurons comparing contralateral and ipsilateral tau pathology of Sup35NM seeded mice.

	Mean contralateral (n per group)	**Mean ipsilateral (n per group)**	**P value Ipsilateral versus contralateral**
Mean AT8 positive neurons in CA3, ± SD	22.07 ± 8.06 (n=7)	35.55 ± 15.70 (n=7)	p= 0.013
Mean Gallyas-Braak positive neurons in CA3, ± SD	14.54 ± 7.15 (n=9)	28.18 ± 13.92 (n=9)	p= 0.002

Table S3

Average age of seeded mice at perfusion per group.

	B6-bh	**-5TyrSup35NM**	**Sup35NM**
Months	6.61 ± 0.56 (n=10)	6.19 ± 0.40 (n=9)	6.15 ± 0.50 (n=9)

2.2 Manuscript No. 2

Severe oligomeric tau toxicity can be reversed without long-term sequelae

Alfonso Martinisi (Alfonso.Martinisi@usb.ch)[1, 2, *], Martin Flach (Martin.Flach@usb.ch)[1, 2, *], Frederik Sprenger (freddy.sprenger@gmail.com)[1, 2, *], Stephan Frank (Stephan.Frank@usb.ch)[1], Markus Tolnay (Markus.Tolnay@usb.ch)[1], David T. Winkler (David.Winkler@usb.ch)[1, 2, 3]

[1]Institute of Medical Genetics and Pathology, and [2]Department of Neurology, University Hospital Basel, Petersgraben 4, CH-4031 Basel, Switzerland, [3]Neurology, Medical University Clinic, Kantonsspital Baselland, Rheinstrasse 26, 4410 Liestal, Switzerland

[*] Contributed equally to this work

Short title: Long-term fitness after tauopathy recovery

Correspondence to:

D.T. Winkler, Institute of Medical Genetics and Pathology, and Dept. of Neurology, University Hospital Basel, Petersgraben 4, CH-4031 Basel, Switzerland

Tel: 0041 61 328 61 62; Fax: 0041 61 265 41 00

Email: David.Winkler@usb.ch

Abstract

Tau is a microtubule stabilizing protein that forms abnormal aggregates in many neurodegenerative disorders, including Alzheimer's disease. We have previously shown that co-expression of fragmented and full-length tau in P301SxTAU62^{on} tau transgenic mice results in the formation of oligomeric tau species and causes severe paralysis. This paralysis is fully reversible once expression of the tau fragment is halted, even though P301S tau expression is maintained. Whereas various strategies to target tau aggregation have been developed, little is known about the long-term consequences of reverted tau toxicity.

Therefore, we studied the long-term motor fitness of recovered, formerly paralyzed P301SxTAU62$^{on\text{-}off}$ mice. To further assess the seeding competence of oligomeric toxic tau species, we also inoculated ALZ17 mice with brainstem homogenates from paralyzed P301SxTAU62^{on} mice.

Counterintuitively, after recovery from paralysis due to oligomeric tau species expression, aging P301SxTAU62$^{on\text{-}off}$ mice did not develop more motor impairment or tau pathology when compared to heterozygous P301S tau transgenic littermates. Thus, toxic tau species causing extensive neuronal dysfunction can be cleared without inducing seeding effects. Moreover, these toxic tau species also lack long-term tau seeding effects upon intrahippocampal inoculation into ALZ17 mice.

In conclusion, tau species can be neurotoxic in the absence of seeding-competent tau aggregates, and mice can clear these tau forms permanently without tau seeding or spreading effects. These observations suggest that early targeting of non-fibrillar tau species may represent a therapeutically effective intervention in tauopathies. On the other hand, the absent seeding competence of early toxic tau species also warrants caution when using seeding-based tests for preclinical tauopathy diagnostics.

Key words: Alzheimer's disease; Neurodegeneration; Tau; Seeding; Oligomers

Abbreviations

AD: Alzheimer’s disease

NFT: Neurofibrillary tangles

H&E: Hematoxylin and eosin

HRP: Horseradish peroxidase

ANOVA: Analysis of variance

AgD: Argyrophilic grain disease

PSP: Progressive supranuclear palsy

CBD: Corticobasal degeneration

Introduction

Tau is a soluble protein acting as a microtubule stabilizer in neuronal cells. Under pathological conditions it becomes hyperphosphorylated and eventually forms intracellular aggregates. Tau aggregates are a hallmark of Alzheimer's disease (AD) and other neurodegenerative disorders, including forms of frontotemporal dementia (Spillantini and Goedert, 2013; Spires-Jones and Hyman, 2014). The mechanisms underlying pathological tau aggregation remain only partly understood. Tau is thought to aggregate through the formation of oligomeric species and subsequent aggregation into fibrils, culminating in the formation of tangles. Tau aggregation can spread towards anatomically connected regions in a prion-like manner (Clavaguera *et al.*, 2009; Goedert *et al.*, 2017).

We have recently shown that tau toxicity can be mediated by oligomeric tau species (Ozcelik *et al.*, 2016). Co-expression of full-length P301S mutant tau with a 3R $tau_{151-421}$ fragment ($\Delta tau_{151-421}$) in 3-weeks-old P301SxTAU62^{on} transgenic mice leads to the formation of soluble high molecular weight tau oligomers. In the P301SxTAU62^{on} mouse model, high molecular weight tau oligomers are sufficient to cause extensive nerve cell dysfunction and severe motor palsy, both of which occur in the absence of insoluble tau aggregates or neurofibrillary tangles (NFTs) (Ozcelik *et al.*, 2016). Strikingly, once the doxycycline-inducible expression of $\Delta tau_{151-421}$ is switched off in these mice, their severe phenotype reverses within 3 weeks, and animals regain their motor competence, even though heterozygous P301S mutant tau expression is maintained (Ozcelik *et al.*, 2016). This phenotype reversibility renders P301SxTAU62$^{on\text{-}off}$ mice suitable for investigating potential long-term consequences of reverted tau toxicity. The relevance of oligomeric tau toxicity is being increasingly recognized, and recent studies have shown that it can also be pharmacologically attenuated (Bittar *et al.*, 2019; Lo *et al.*, 2019; Abskharon *et al.*, 2020; Lo Cascio *et al.*, 2020; Puangmalai *et al.*, 2020). Hypothesized to depend on oligomer conformation, tau oligomeric toxicity can also be aggravated by biological interactions with chaperones (Oroz *et al.*, 2018), RNA-binding proteins (Jiang *et al.*, 2019), and nuclear complexes (Eftekharzadeh *et al.*, 2018). Due to their interaction with cellular components and their importance in tau propagation, tau oligomers have been considered crucial for tau-mediated neurodegeneration.

Long-term effects after recovery from oligomeric, non-fibrillar tau toxicity have not yet been assessed. Only few studies, using inducible tau mouse models, have analyzed the reversibility of tau tangle pathology

(Santacruz *et al.*, 2005; Polydoro *et al.*, 2013). Here, we study the late effects of reverted tau stress in aged P301SxTAU62$^{on\text{-}off}$ mice. Seeding and spreading of tau pathology have been widely studied in human tauopathies as well as in tau transgenic mouse models (Maeda *et al.*, 2007; Clavaguera *et al.*, 2009; Sanders *et al.*, 2014; Mudher *et al.*, 2017). Based on this accumulated evidence, we hypothesized that the tau oligomers abundantly present in young paralyzed P301SxTAU62^{on} mice might act as seed for tau accumulation, as P301S full-length tau expression is maintained, while doxycycline-driven $\Delta tau_{151\text{-}421}$ expression is switched off. To our surprise, P301SxTAU62$^{on\text{-}off}$ mice that had recovered from neurotoxic stress and motor palsy did not develop worse motor function nor more pronounced tau pathology during aging when compared to their P301S tau heterozygous littermates. This demonstrates that P301SxTAU62$^{on\text{-}off}$ mice clear their toxic tau aggregates without seeding NFT formation, and hence do not show pronounced motor decline upon aging.

To study whether the toxic tau species causing palsy in young P301SxTAU62^{on} mice also lack classical seeding competence, we next stereotactically inoculated brainstem homogenates of paralyzed P301SxTAU62^{on} into ALZ17 human wild-type tau transgenic mice. In contrast to ALZ17 mice seeded with P301S brainstem homogenates containing fibrillary tau species (Clavaguera *et al.*, 2009), ALZ17 mice inoculated with P301SxTAU62^{on} homogenate did not develop fibrillar tau pathology, arguing against a seeding competence of the early toxic tau forms in P301SxTAU62^{on} mice.

Lacking seeding competence of non-fibrillar toxic tau species as well as absent long-term sequelae after halting their expression warrants the exploration of early therapeutic interventions targeting pre-fibrillar tau species. Our observations may also question the reliability of seeding-based assays for early preclinical tauopathy diagnostics.

Methods

Mice

Transgenic homozygous mice expressing human ALZ17 mutant tau (ALZ17 mice) (Probst *et al.*, 2000), transgenic homozygous mice expressing human P301S mutant tau (P301S mice) (Allen *et al.*, 2002), transgenic heterozygous mice expressing human $\Delta tau_{151\text{-}421}$ (TAU62 mice) (Ozcelik *et al.*, 2016), and non-transgenic C57BL/6J (BL6 mice) control mice were used. P301S mice and TAU62 mice were interbred to obtain double transgenic P301SxTAU62 mice (Ozcelik *et al.*, 2016). All animal experiments were performed in compliance with protocols approved by the Committee for Animal Care and Animal Use of the Canton of Basel (Licenses Nr. BS 2364 and 2471).

Behavioral tests

Motor behavior, including gait ataxia, tremor, and hindlimb reflexes was assessed. Quantitative motor testing was performed by the grid test in which mice were placed on a vertical mesh grid and the latency to fall off the grid was recorded for 3 min. Motor coordination and balance were assessed using the Panlab Harvard Rotarod (Harvard Apparatus, Holliston, USA). The rotarod starts at a speed of 4 rpm and accelerates by 1 rpm every 3 sec. In both, grid test and rotarod assay, mice were tested for 3 consecutive days with 3 trials per day, with minimum rest intervals of 5 min, and the mean latency to fall was documented. Results were obtained by averaging the daily means of 3 consecutive days.

Stereotactic surgery

For surgery, 3-months-old ALZ17 mice were anesthetized with a mixture of ketamine (10 mg/kg) and xylazine (20 mg/kg) and placed on a heating pad to maintain body temperature. Mice were stereotactically injected into the right hippocampus (A/P, −2.5 mm from bregma; L, − 2.0 mm; D/V, −1.8 mm) using a Hamilton syringe. Brainstem homogenates of 6-months-old paralyzed homozygous P301S mice and 3-weeks-old paralyzed P301SxTAU62^{on} mice were prepared for inoculation in ALZ17 mice. Brainstems were weighted and diluted 1:10 in PBS for seeding. After dilution, samples were homogenized using an Ultraturrax T8 (IKA Labortechnik, Staufen im Breisgau, Germany) and sonicated briefly (Bandelin SONOPULS, Bandelin, Berlin, Germany; 90% power, 10% cycle, 10 sec pulses). Homogenates were then centrifuged at 4000 g for 20 minutes

at 4 °C, and aliquots of the supernatant were stored at -70 °C for later usage. During inoculation, each mouse received 5 µl of brainstem homogenate at a speed of 1.25 µl/min. Following injection, the needle was kept in place for additional 3 minutes before withdrawal. The surgical area was cleaned with sterile saline and the incision sutured. Mice were monitored until recovery from anaesthesia, post-interventional analgesia was administered, and animals were checked regularly following surgery. After 20 months of incubation, the seeded mice were sacrificed.

Immunohistochemistry

Mice were deeply anesthetized with pentobarbital and transcardially perfused with 20 ml cold PBS, followed by 20 ml 4% paraformaldehyde in PBS. The brains were dissected and post-fixed overnight. Following paraffin embedding, 4 µm coronal sections were cut from the brains of seeded mice, whereas 4 µm sagittal sections were prepared from the brains of mice used for the behavioral tests. Sections were silver-impregnated following the method of Gallyas-Braak to visualize filamentous tau pathology. Hematoxylin and eosin staining (H&E) was performed for morphological analysis. For immunohistochemistry, the following anti-tau antibodies were used: AT8 (1:1000, Pierce Biotechnology, Waltham, USA), AT100 (1:1000, Pierce Biotechnology, Waltham, USA), TauC3 (1:1000, Santa Cruz Biotechnology, Santa Cruz, USA). Secondary antibodies were from Vector Laboratories, Burlingame, USA (Vectastain ABC kit). 10X, 20X and 40X magnified pictures of the stained slides were taken with an Olympus BX43 Upright Microscope (Life Sciences Solutions, Chicago, USA).

Western blots

For the western blots comparing total and soluble tau in 16-months-old P301S heterozygous (P301Shet) and P301SxTAU62$^{on\text{-}off}$ mice, brains were homogenized in cold extraction buffer 20 % (w/v) (25 mM Tris-HCl pH 7.4, 150 mM NaCl, 1mM EDTA, 1mM EGTA, 5 mM NaPyrophosphate, 10 mM B Glycerophosphate, 30 mM NaFluoride, 10 mM NaVanadate, with addition of 100 ul/10 ml PMSF of 0.1 M just before use, and 1 Pierce protease and phosphatase inhibitor mini tablet, EDTA-free, Pierce Biotechnology, Waltham, USA) using a Polytron Homogenizer (Thomas Scientific). An aliquot of the resulting homogenate was collected as total tau. Subsequently, samples were centrifuged at 80000 g for 15 min using an ultracentrifuge (Beckman Coulter, Brea, USA; OptimaTM L-70K Ultracentrifuge), and an aliquot of the supernatant was collected as soluble tau.

For the western blot comparing total tau in 3-months-old mice, one half of the mouse brain was dissected into forebrain and brainstem, and frozen in liquid nitrogen or on dry ice. Brainstems were weighted and diluted 1:10 in TBS-Complete. Subsequently, samples were homogenized using an Ultraturrax T8 (IKA Labortechnik, Staufen im Breisgau, Germany) and briefly sonicated (Bandelin SONOPULS, Bandelin, Berlin, Germany; 90% power, 10% cycle, 10 sec pulses). Homogenates were then centrifuged at 4000 g for 20 minutes at 4 °C, and aliquots of the supernatant stored at -70 °C for later usage. Western blots were then performed under non-reducing conditions using appropriate amounts of protein, 4X NuPAGE LDS sample buffer, and deionized water. 10X NuPAGE reducing agent was used to obtain reducing conditions. Following appropriate preparation, samples were loaded onto a 7% NuPAGE® Tris-acetate gel. After the removal of gels from the cassette and activation of PVDF membrane (Amersham Biosciences, Amersham, UK) samples were transferred on the PVDF membrane using the XCell IITM Blot Module. Unspecific binding epitopes were blocked with 5% non-fat milk in PBS-Tween, followed by incubation with primary antibody over night at 4 °C on a shaker. After washing with PBS-Tween, the membrane was incubated with horseradish peroxidase (HRP)-conjugated anti-mouse or -rabbit secondary antibody at room temperature. Then, the membrane was washed again in PBS-Tween and detected by electrochemiluminescence (ECL) (GE Healthcare, Little Chalfont, UK). The anti-tau antibody used for the western blots was HT7 (1:1000, Pierce Biotechnology, Waltham, USA).

Dot blots

For dot blots, serum of 3-months-old P301Shet and P301SxTAU62$^{on\text{-}off}$ mice was separated from the clot by centrifuging the samples at 1000 rpm for 15 minutes at 4 °C, with the remaining supernatant aliquoted and stored at -20 °C, for later usage. Then, a nitrocellulose membrane (Bio-Rad Laboratories, Inc., Hercules, USA) was divided in a grid to allow later incubations with 3-months-old P301Shet and P301SxTAU62$^{on\text{-}off}$ sera and HT7 antibody as positive control at 1:1000 dilutions. 0.2 mg/ml of 2N4R wild-type tau monomers were applied on a 4x3 grid. Next, unspecific binding epitopes were blocked with 5% BSA in PBS-Tween, followed by a 30-minutes-long incubation at room temperature with previously extracted sera from the mice and HT7 antibody as positive control. After washing with PBS-Tween, the membrane was incubated with HRP-conjugated anti-mouse secondary antibody at room temperature, washed again in PBS-Tween, and detected by ECL (GE Healthcare, Little Chalfont, UK).

Statistical analysis

To evaluate behavioral test results statistically, one-way analysis of variance (ANOVA) followed by post-hoc Student's t-tests and Bonferroni correction for multiple comparisons were applied. P-values < 0.05 were considered significant. To estimate soluble tau and total tau expression from western blots of 3-months-old and 16-months-old mice, the protein bands were normalized to GAPDH protein standard, and quantified using ImageJ software; generated mean ratio values were compared by Student's t-test. To determine the effect of early neurotoxic stress in aged mice, AT8 and Gallyas-positive neurons were semi-quantitatively assessed in brainstem regions. Three sections per animal were analyzed. The total area analyzed per animal was comparable between animals (AT8: P-value = 0,34, and Gallyas: P-value = 0,91). The average count/area ratios were obtained. P-values calculated by Student's t-tests were interpreted exploratory and not adjusted for multiple comparisons; P-values < 0.05 were considered significant. Box plots for figures 2 to 5 were generated with R software. The lower and upper hinges correspond to the first and third quartiles (25th and 75th percentiles). The upper whisker extends from the hinge to the largest value no further than 1.5 times IQR from the hinge (where IQR is the inter-quartile range, or distance between the first and third quartiles). The lower whisker extends from the hinge to the smallest value at most 1.5 times IQR of the hinge. Data beyond the end of the whiskers are called "outlying" points and are plotted individually.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Results

Co-expression of tau fragment and full-length tau induces severe reversible neurotoxicity in P301SxTAU62^{on} mice despite the absence of tau fibrils

P301SxTAU62^{on} transgenic mice co-expressing human $\Delta tau_{151-421}$ with full-length P301S mutant tau were obtained by interbreeding P301S tau transgenic mice with TAU62 transgenic mice, where $\Delta tau_{151-421}$ expression is regulated by a doxycycline-responsive promoter element (Ozcelik *et al.*, 2016). 3-weeks-old heterozygous P301S transgenic littermates (P301Shet) did not exhibit signs of motor dysfunction, as also illustrated by their normal tail suspension test (Fig. 1A). In contrast, P301SxTAU62^{on} mice of the same age showed a severe hindlimb palsy, only being able to move by the use of their forelimbs (Fig. 1B). Upon halting doxycycline administration to stop $\Delta tau_{151-421}$ expression, this phenotype was fully reversible within three weeks, even though P301S tau expression continued (Fig. 1C).

Histological examination did not reveal any tau pathology in young P301Shet mice (Fig. 1A). In contrast, extensive AT8-positive pre-tangle stage tau pathology was found in paralyzed, 3-weeks-old P301SxTAU62^{on} mice (Fig. 1B). Remarkably, three weeks after $\Delta tau_{151-421}$ expression had been stopped, AT8-positive tau pathology was no longer detectable, and by the age of six weeks, P301SxTAU62^{on-off} mice had regained normal walking capability (Fig. 1C). Gallyas-Braak silver stain positive tau pathology was absent in 3-weeks-old P301Shet mice (Fig. 1A), their paralyzed P301SxTAU62^{on} littermates (Fig. 1B), as well as in recovered P301SxTAU62^{on-off} mice (Fig. 1C, for high magnification pictures see Supplementary Fig. 1). These observations confirm that the severe, but reversible motor palsy in P301SxTAU62^{on-off} mice is mediated by non-fibrillar, Gallyas-Braak silver stain-negative tau species (Ozcelik *et al.*, 2016). A graphical representation of these findings is given in Supplementary Fig. 2.

Absence of excessive motor impairment in aged P301SXTAU62^{on-off} mice after recovery from early severe neurotoxicity

To study the long-term effects of the severe non-fibrillar tau stress in P301SxTAU62^{on} mice, we followed their motor capabilities after their initial recovery. As only $\Delta tau_{151-421}$ expression is under the control of a

doxycycline-responsive promoter element, recovered P301SxTAU62$^{on\text{-}off}$ mice maintain expression of P301S mutant full-length tau.

We compared formerly paralyzed, recovered and aged P301SxTAU62$^{on\text{-}off}$ mice to their P301Shet littermates and to non-transgenic BL6 mice by tail suspension, rotarod, and grid tests at the age of 16 months. P301Shet littermates showed signs of hindlimb clasping, while recovered P301SxTAU62$^{on\text{-}off}$ mice were still able to spread their hindlimbs, as were non-transgenic BL6 mice (Fig. 2A).

As expected, at 16 months of age, motor balance (rotarod) was significantly reduced in both tau expressing mouse models, compared to non-transgenic BL6 mice. Somewhat surprisingly, however, we did not detect a worse motor performance of P301SxTAU62$^{on\text{-}off}$ mice in comparison to their P301Shet littermates. In contrast, formerly paralyzed P301SxTAU62$^{on\text{-}off}$ mice performed even slightly better in the rotarod test when compared to P301Shet littermates, although this did not reach statistical significance (Fig. 2B). Similar observations were made for the grid test, revealing a significantly reduced motor strength in P301Shet transgenic mice in comparison to non-transgenic BL6 mice. Again, at the age of 16 months, P301SxTAU62$^{on\text{-}off}$ mice showed a slightly better grid test performance than their P301Shet littermates, although again without statistical significance (Fig. 2C, see Supplementary Table 1 for complete values). Thus, P301SxTAU62$^{on\text{-}off}$ mice recovered from early neurotoxic tau stress and did not show increased motor impairment with aging when compared to P301Shet littermates. These findings are graphically summarized in Supplementary Fig. 2.

Absence of excessive tau pathology in aged P301SxTAU62$^{on\text{-}off}$ mice after recovery from severe neurotoxicity

We next aimed at studying whether the absence of a pronounced motor phenotype in P301SxTAU62$^{on\text{-}off}$ mice would also be mirrored by the extent of tau pathology upon aging. To this end, we comparatively analyzed brains of 16-months-old P301SxTAU62$^{on\text{-}off}$ and P301Shet mice by histology. In all mice, tau pathology was most extensive in the brainstem. AT8 immunohistochemistry revealed extensive tau hyperphosphorylation in P301Shet mice whereas significantly less tau hyperphosphorylation was present in P301SxTAU62$^{on\text{-}off}$ animals (Fig. 3A, B, for high magnification pictures see Supplementary Fig. 3). Additional immunohistochemistry with the AT100 antibody revealed similar results for 16-months-old mice, confirming the significantly more

abundant hyperphosphorylation in the heterozygous mice which did not experience the early neurotoxic stress as the recovered mice did (see Supplementary Fig. 4).

Gallyas-Braak silver staining revealed widespread, robust tau tangle formation in the brainstem of P301Shet mice, whereas formerly paralyzed P301SxTAU62$^{on\text{-}off}$ mice remained almost devoid of fibrillar tau pathology (Fig. 3C, D, for high magnification pictures see Supplementary Fig. 3). Semiquantitative assessment confirmed the lower extent of tau pathology in aged P301SxTAU62$^{on\text{-}off}$ compared to P301Shet mice (see Supplementary Table 2 for complete values). Immunohistochemistry with TauC3 antibody did not reveal leakage of $\Delta tau_{151\text{-}421}$ expression in 16-months-old P301SxTAU62$^{on\text{-}off}$ mice (Supplementary Fig. 4). A graphical representation of these findings can be found in Supplementary Fig. 2.

We confirmed our histological findings by western blotting, using the human tau targeting HT7 antibody. Significantly higher levels of total (Fig. 4A, B) and soluble tau (Fig. 4C, D) were detected in P301Shet mice compared to P301SxTAU62$^{on\text{-}off}$ mice at 16 months of age (see Supplementary Table 2 for complete values). Next, we wanted to rule out that the mild motor phenotype as well as the comparatively mild tau pathology in aged P301SxTAU62$^{on\text{-}off}$ mice were caused by loss of human mutant P301S tau expression. To this end, we analyzed tau protein levels in these mice and their heterozygous littermates at the age of 3 months. Western blot analysis revealed comparable total tau levels in P301Shet and P301SxTAU62$^{on\text{-}off}$ mice (Fig. 5A, B; see Supplementary Table 2 for complete values). It is therefore unlikely, that the maintained motor competence of aged P301SxTAU62$^{on\text{-}off}$ mice occurs despite a potential previous loss of tau-expressing neurons in young paralyzed P301SxTAU62^{on} mice. Nevertheless, based on Western blotting, discretely reduced tau expression levels in P301SxTAU62$^{on\text{-}off}$ compared to P301Shet mice cannot be excluded. Therefore, a mildly lowered tau expression might have been contributed to the slightly better motor performance and the less extensive tau pathology in aged P301SxTAU62$^{on\text{-}off}$ mice. In light of the extensive early tau toxicity occurring in P301SxTAU62^{on} mice, an immunological reaction could also have been triggered by the toxic high molecular weight tau, and may have caused the lower tau levels in aged P301SxTAU62$^{on\text{-}off}$ mice. However, dot blot analysis did not detect any anti-tau antibodies in sera of 3-months-old P301Shet and P301SxTAU62$^{on\text{-}off}$ mice (Supplementary Fig. 5).

Brainstem homogenates from paralyzed P301SxTAU62^{on} mice do not induce NFT formation in ALZ 17 mice

Our observations made so far argued against a long-term seeding effect of the early, non-fibrillar neurotoxic tau species which caused the severe motor palsy in P301SxTAU62^{on} mice. To assess the seeding capacity of these non-fibrillar toxic tau species also in a classical tau seeding setting (Clavaguera *et al.*, 2009), we next prepared brainstem homogenates from paralyzed 3-weeks-old P301SxTAU62^{on} mice, and inoculated them intrahippocampally into 3-months-old ALZ17 tau transgenic mice. Brain homogenates derived from aged tangle-bearing homozygous P301S tau transgenic mice were used as positive controls, as these had previously been proven to provoke the formation of Gallyas-Braak silver stain-positive tangles when inoculated into ALZ17 mice (Clavaguera *et al.*, 2009). As expected, ALZ17 mice seeded with P301S brainstem homogenates developed granular focal tau pathology, primarily in CA1 (Fig. 6A) and the ipsilateral dorsal fornix above CA1 (Fig. 6B). In contrast, ALZ17 mice seeded with P301SxTAU62^{on} brainstem homogenates remained devoid of Gallyas-Braak silver stain positive structures up to the age of 20 months. This observation indicates that the non-fibrillar toxic tau species associated with the severe paralysis of P301SxTAU62^{on} mice are not seeding-competent. A graphical representation of these findings is given in the Supplementary Fig. 6.

Discussion

We here report that early neurotoxic tau stress causing severe motor paralysis in P301SxTAU62^{on} mice is fully reversible and does not cause late seeding or adverse effects in recovered P301SxTAU62$^{on\text{-}off}$ mice during aging. Furthermore, we find that the inoculation of brainstem homogenates from paralyzed P301SxTAU62^{on} mice into ALZ17 mice does not result in tau seeding in the host mice, demonstrating that non-fibrillar neurotoxic tau species can lack classical seeding competence.

Co-expression of a $\Delta tau_{151\text{-}421}$ with full-length P301S mutant tau in P301SxTAU62^{on} mice causes severe paralysis after three weeks, as we have previously reported (Ozcelik *et al.*, 2016). This motor impairment is paralleled by an increase in hyperphosphorylated tau, but occurs in the absence of fibrillar tau forms or tau tangles. Upon switching off $\Delta tau_{151\text{-}421}$ expression, hyperphosphorylated tau species are no longer detectable and P301SxTAU62$^{on\text{-}off}$ mice regain their motor function. This confirms that hyperphosphorylated non-fibrillar tau oligomers can mediate early neurotoxicity, and that clearance of these tau species rescues the paralyzed mice from their motor impairment (Ozcelik *et al.*, 2016).

While over-expression of non-aggregating tau deletion mutants lacks relevant neurotoxicity (Macdonald *et al.*, 2019), we here confirm that soluble oligomeric, yet non-fibrillar tau aggregates can exert extensive tau toxicity. This is also in line with reported clinical data: tau oligomers appear early in the brains of patients developing AD (Patterson *et al.*, 2011; Lasagna-Reeves *et al.*, 2012; Koss *et al.*, 2016) and progressive supranuclear palsy (Gerson *et al.*, 2014), possibly even before first clinical symptoms become apparent (Maeda *et al.*, 2006). Tau oligomers have also been shown to correlate well with neurodegeneration in tau transgenic mice (Gomez-Isla *et al.*, 1997; Lasagna-Reeves *et al.*, 2011; Jiang *et al.*, 2019). Based on this, oligomeric tau species are now considered to play a central role in the pathogenesis of neuronal dysfunction in tauopathies (Ghag *et al.*, 2018; Polanco *et al.*, 2018; Sengupta *et al.*, 2018; Bittar *et al.*, 2019), comparably to oligomeric structures in other neurodegenerative disorders (Hong *et al.*, 2018; Sekiya *et al.*, 2019). The severe early neurotoxic stress provoked by non-fibrillar tau forms in our P301SxTAU62$^{on\text{-}off}$ mice strengthens this view.

Counterintuitively, we here find that P301SxTAU62$^{on\text{-}off}$ mice, which have been exposed to non-fibrillar neurotoxic tau, do not show an accelerated course of their tauopathy during aging. In contrast, aged P301SxTAU62$^{on\text{-}off}$ mice show rather better motor performance and less tau pathology in comparison to their

heterozygous P301S littermates, even though P301S tau expression remains unaffected upon switching off $\Delta tau_{151\text{-}421}$ expression. To our knowledge, this is the first analysis of long-term consequences of early tau stress in a model maintaining the expression of an aggregation-prone tau form. Previous studies were conducted in mouse models conditionally expressing a single mutant human tau form, and the recovery of these mice was achieved by its suppression (Van der Jeugd *et al.*, 2012; Polydoro *et al.*, 2013; Wang *et al.*, 2018). Thus, upon switch-off, aggregation-prone human tau forms were no longer expressed in these mice – a scenario, which does not reflect the situation of potential interventional tau clearance in man. In addition, previous studies in rTgTauEC mice have shown that NFT-associated toxicity can be reversed by suppressing tau overexpression (Polydoro *et al.*, 2013). In pro-aggregant hTau40 transgenic mice, motor fitness improved after stopping mutant tau expression (Van der Jeugd *et al.*, 2012). Similar results were obtained in PS19 mice by antisense oligonucleotide-mediated downregulation of mutant tau expression (DeVos *et al.*, 2013; DeVos *et al.*, 2017).

An early study in rTg4510 mice has shown that tau tangle formation can be dissociated from neuronal dysfunction (Santacruz *et al.*, 2005). In particular, cognitive functions recovered once expression of transgenic P301L tau had been halted, but tau tangles continued to accumulate (Santacruz *et al.*, 2005). Our present findings now further separate tau toxicity from tangle formation by providing first evidence that early tau toxicity can be reverted even without subsequent seeding of fibrillar tau tangles. This indicates that halting tau oligomer-related toxicity prior to the appearance of filamentous tau can not only reverse clinical symptoms (as reflected by the motor phenotype recovery of our mice), but may also efficiently prevent long-term tau seeding and spreading. Strikingly, interventions with compounds targeting tau oligomers have already shown beneficial effects, including the reversal of brain dysfunction (Castillo-Carranza *et al.*, 2014; Soeda *et al.*, 2015; Gerson *et al.*, 2018; Lo Cascio *et al.*, 2019; Lo *et al.*, 2019). Our new findings suggest that early therapeutic tau oligomer removal might efficiently prevent the progression of tauopathies.

We cannot exclude that the lack of a late tau seeding effect by early toxic tau species in aging P301SxTAU62$^{on\text{-}off}$ mice is caused by immunological reactions targeting tau in these mice. The slower decline of motor function of recovered, aging P301SxTAU62$^{on\text{-}off}$ mice could also be associated with a protective mechanism triggered by the early neurotoxic tau stress. While a dot blot analysis of sera of P301SxTAU62$^{on\text{-}off}$ mice did not reveal any antibodies targeting recombinant tau, this does not fully rule out alternative immunological mechanisms (e.g. T-cell mediated; antibodies against specific epitopes of oligomeric tau assemblies). Furthermore, the

slightly lowered tau levels in aged P301SxTAU62$^{on\text{-}off}$ mice could also be a consequence of autophagy induction by the early oligomeric tau stress that these mice experienced; in support of the latter notion, it was previously found that pharmacological autophagy activation can defer tau pathology progression in P301S mice (Schaeffer *et al.*, 2012; Ozcelik *et al.*, 2013).

As we cannot exclude that the lack of a late tau seeding effect by early toxic tau species in P301SxTAU62$^{on\text{-}off}$ mice is attributable to immunological reactions or the induction of the autophagy pathway targeting oligomeric tau in these mice, we next investigated whether the early toxic tau species exert classical prion-like seeding competence upon intracerebral inoculation. Fibrillar tau induces the aggregation of soluble tau species in a prion-like manner upon inoculation into tau transgenic host mice (Clavaguera *et al.*, 2009; Goedert *et al.*, 2017). We inoculated ALZ17 mice with brainstem homogenates from paralyzed P301SxTAU62^{on} mice. These seeded ALZ17 mice remained completely free of fibrillar tau pathology up to high ages. In contrast, seedings with P301S brainstem homogenates induced focal granular Gallyas-Braak silver stain-positive tau aggregates, as these homogenates also contain short tau filaments, known to be the most seeding-competent tau species (Jackson *et al.*, 2016). The absence of fibril induction in ALZ17 mice inoculated with brainstem homogenates from paralyzed P301SxTAU62^{on} mice confirms that early toxic tau oligomers can lack fibrillar tau seeding competence.

At late disease stages, seeding-competent tau structures are present in most tauopathies, and capable of seeding fibrillar tau aggregates when injected into ALZ17 mice (Clavaguera *et al.*, 2013). Interestingly, the only homogenates not provoking the formation of Gallyas-positive fibrils in ALZ17 mice, comparable to our P301SxTAU62^{on} brainstem homogenates, were extracts collected from argyrophilic grain disease (AgD) patients. This has been linked to the histological predominance of pre-tangles over fully formed tangles in AgD (Ferrer *et al.*, 2008), similar to the pre-tangle stage pathology present in our paralyzed P301SxTAU62^{on} mice. A low seeding activity of AgD extracts has recently also been found in an RT-QuIC *in vitro* seeding model (Kraus *et al.*, 2019). In that study, also brain extracts from other tauopathies exerted only low seeding effects, demonstrating the presence of non-, or not-yet seeding-competent tau forms in human tauopathies including progressive supranuclear palsy (PSP) and corticobasal degeneration (CDB) (Kraus *et al.*, 2019). A comparably low seeding efficacy of PSP and CDB homogenates was found in a cell-based aggregation assay, while seeds of AD patients resulted in high tau aggregation (Chung *et al.*, 2019). In this light, our present findings warrant

caution when developing aggregation and seeding-based diagnostic assays. Very early tau forms could be toxic, but undetectable by seeding-based diagnostic tools.

In conclusion, we here demonstrate that high molecular weight tau oligomers can provoke a severe, but reversible neurotoxicity. Rescued mice do not develop long-term sequelae, which argues for early therapeutic interventions targeting oligomeric tau forms. Furthermore, we confirm that these early toxic tau species lack classical seeding competence. Therefore, caution should be exercised when using seeding-based assays for the detection of very early preclinical tauopathy manifestations. Our findings also warrant a deepened analysis of early, non-fibrillar toxic tau forms in the future. While the knowledge on filamentous tau strains is rapidly growing thanks to Cryo-EM studies in particular (Fitzpatrick *et al.*, 2017; Falcon *et al.*, 2018a; Falcon *et al.*, 2018b; Falcon *et al.*, 2019), the puzzling characteristics of non-, or not-yet fibrillar tau forms remain only incompletely understood.

Acknowledgments

We thank Michel Goedert and Jennifer MacDonald from the MRC Laboratory of Molecular Biology, Cambridge (UK), for the helpful discussions and the technical support.

Funding

MT and DTW are supported by the Swiss National Science Foundation (31003A_152846 to MT and 32323B_123812 and 310030_169486 to DTW), and the Synapsis Foundation Switzerland

Competing interests

The authors declare no competing interests.

Author notes

DTW designed the study; AM, MF, FS and DTW designed research; AM, MF and FS performed research; AM, MF, MT, and DTW analysed data; and AM, SF and DTW wrote the paper. AM, MF and FS contributed equally to the manuscript. All authors read and approved the final manuscript.

References

Abskharon R, Seidler PM, Sawaya MR, Cascio D, Yang TP, Philipp S*, et al.* Crystal structure of a conformational antibody that binds tau oligomers and inhibits pathological seeding by extracts from donors with Alzheimer's disease. J Biol Chem 2020; 295(31): 10662-76.

Allen B, Ingram E, Takao M, Smith MJ, Jakes R, Virdee K*, et al.* Abundant tau filaments and nonapoptotic neurodegeneration in transgenic mice expressing human P301S tau protein. J Neurosci 2002; 22(21): 9340-51.

Bittar A, Bhatt N, Hasan TF, Montalbano M, Puangmalai N, McAllen S*, et al.* Neurotoxic tau oligomers after single versus repetitive mild traumatic brain injury. Brain Commun 2019; 1(1): fcz004.

Castillo-Carranza DL, Sengupta U, Guerrero-Munoz MJ, Lasagna-Reeves CA, Gerson JE, Singh G*, et al.* Passive immunization with Tau oligomer monoclonal antibody reverses tauopathy phenotypes without affecting hyperphosphorylated neurofibrillary tangles. J Neurosci 2014; 34(12): 4260-72.

Chung DC, Carlomagno Y, Cook CN, Jansen-West K, Daughrity L, Lewis-Tuffin LJ*, et al.* Tau exhibits unique seeding properties in globular glial tauopathy. Acta neuropathologica communications 2019; 7(1): 36.

Clavaguera F, Akatsu H, Fraser G, Crowther RA, Frank S, Hench J*, et al.* Brain homogenates from human tauopathies induce tau inclusions in mouse brain. Proc Natl Acad Sci U S A 2013; 110(23): 9535-40.

Clavaguera F, Bolmont T, Crowther RA, Abramowski D, Frank S, Probst A*, et al.* Transmission and spreading of tauopathy in transgenic mouse brain. Nat Cell Biol 2009; 11(7): 909-13.

DeVos SL, Goncharoff DK, Chen G, Kebodeaux CS, Yamada K, Stewart FR*, et al.* Antisense reduction of tau in adult mice protects against seizures. J Neurosci 2013; 33(31): 12887-97.

DeVos SL, Miller RL, Schoch KM, Holmes BB, Kebodeaux CS, Wegener AJ*, et al.* Tau reduction prevents neuronal loss and reverses pathological tau deposition and seeding in mice with tauopathy. Sci Transl Med 2017; 9(374).

Eftekharzadeh B, Daigle JG, Kapinos LE, Coyne A, Schiantarelli J, Carlomagno Y*, et al.* Tau Protein Disrupts Nucleocytoplasmic Transport in Alzheimer's Disease. Neuron 2018; 99(5): 925-40 e7.

Falcon B, Zhang W, Murzin AG, Murshudov G, Garringer HJ, Vidal R*, et al.* Structures of filaments from Pick's disease reveal a novel tau protein fold. Nature 2018a; 561(7721): 137-40.

Falcon B, Zhang W, Schweighauser M, Murzin AG, Vidal R, Garringer HJ*, et al.* Tau filaments from multiple cases of sporadic and inherited Alzheimer's disease adopt a common fold. Acta neuropathologica 2018b; 136(5): 699-708.

Falcon B, Zivanov J, Zhang W, Murzin AG, Garringer HJ, Vidal R*, et al.* Novel tau filament fold in chronic traumatic encephalopathy encloses hydrophobic molecules. Nature 2019; 568(7752): 420-3.

Ferrer I, Santpere G, van Leeuwen FW. Argyrophilic grain disease. Brain 2008; 131(Pt 6): 1416-32.

Fitzpatrick AWP, Falcon B, He S, Murzin AG, Murshudov G, Garringer HJ*, et al.* Cryo-EM structures of tau filaments from Alzheimer's disease. Nature 2017; 547(7662): 185-90.

Gerson JE, Farmer KM, Henson N, Castillo-Carranza DL, Carretero Murillo M, Sengupta U*, et al.* Tau oligomers mediate alpha-synuclein toxicity and can be targeted by immunotherapy. Mol Neurodegener 2018; 13(1): 13.

Gerson JE, Sengupta U, Lasagna-Reeves CA, Guerrero-Munoz MJ, Troncoso J, Kayed R. Characterization of tau oligomeric seeds in progressive supranuclear palsy. Acta neuropathologica communications 2014; 2: 73.

Ghag G, Bhatt N, Cantu DV, Guerrero-Munoz MJ, Ellsworth A, Sengupta U*, et al.* Soluble tau aggregates, not large fibrils, are the toxic species that display seeding and cross-seeding behavior. Protein Sci 2018; 27(11): 1901-9.

Goedert M, Eisenberg DS, Crowther RA. Propagation of Tau Aggregates and Neurodegeneration. Annu Rev Neurosci 2017; 40: 189-210.

Gomez-Isla T, Hollister R, West H, Mui S, Growdon JH, Petersen RC*, et al.* Neuronal loss correlates with but exceeds neurofibrillary tangles in Alzheimer's disease. Ann Neurol 1997; 41(1): 17-24.

Hong W, Wang Z, Liu W, O'Malley TT, Jin M, Willem M*, et al.* Diffusible, highly bioactive oligomers represent a critical minority of soluble Abeta in Alzheimer's disease brain. Acta neuropathologica 2018; 136(1): 19-40.

Jackson SJ, Kerridge C, Cooper J, Cavallini A, Falcon B, Cella CV*, et al.* Short Fibrils Constitute the Major Species of Seed-Competent Tau in the Brains of Mice Transgenic for Human P301S Tau. J Neurosci 2016; 36(3): 762-72.
Jiang L, Ash PEA, Maziuk BF, Ballance HI, Boudeau S, Abdullatif AA*, et al.* TIA1 regulates the generation and response to toxic tau oligomers. Acta neuropathologica 2019; 137(2): 259-77.
Koss DJ, Jones G, Cranston A, Gardner H, Kanaan NM, Platt B. Soluble pre-fibrillar tau and beta-amyloid species emerge in early human Alzheimer's disease and track disease progression and cognitive decline. Acta neuropathologica 2016; 132(6): 875-95.
Kraus A, Saijo E, Metrick MA, 2nd, Newell K, Sigurdson CJ, Zanusso G*, et al.* Seeding selectivity and ultrasensitive detection of tau aggregate conformers of Alzheimer disease. Acta neuropathologica 2019; 137(4): 585-98.
Lasagna-Reeves CA, Castillo-Carranza DL, Sengupta U, Clos AL, Jackson GR, Kayed R. Tau oligomers impair memory and induce synaptic and mitochondrial dysfunction in wild-type mice. Mol Neurodegener 2011; 6: 39.
Lasagna-Reeves CA, Castillo-Carranza DL, Sengupta U, Guerrero-Munoz MJ, Kiritoshi T, Neugebauer V*, et al.* Alzheimer brain-derived tau oligomers propagate pathology from endogenous tau. Scientific reports 2012; 2: 700.
Lo Cascio F, Garcia S, Montalbano M, Puangmalai N, McAllen S, Pace A*, et al.* Modulating Disease-Relevant Tau Oligomeric Strains by Small Molecules. J Biol Chem 2020.
Lo Cascio F, Puangmalai N, Ellsworth A, Bucchieri F, Pace A, Palumbo Piccionello A*, et al.* Toxic Tau Oligomers Modulated by Novel Curcumin Derivatives. Scientific reports 2019; 9(1): 19011.
Lo CH, Lim CK, Ding Z, Wickramasinghe SP, Braun AR, Ashe KH*, et al.* Targeting the ensemble of heterogeneous tau oligomers in cells: A novel small molecule screening platform for tauopathies. Alzheimers Dement 2019; 15(11): 1489-502.
Macdonald JA, Bronner IF, Drynan L, Fan J, Curry A, Fraser G*, et al.* Assembly of transgenic human P301S Tau is necessary for neurodegeneration in murine spinal cord. Acta neuropathologica communications 2019; 7(1): 44.
Maeda S, Sahara N, Saito Y, Murayama M, Yoshiike Y, Kim H*, et al.* Granular tau oligomers as intermediates of tau filaments. Biochemistry 2007; 46(12): 3856-61.
Maeda S, Sahara N, Saito Y, Murayama S, Ikai A, Takashima A. Increased levels of granular tau oligomers: an early sign of brain aging and Alzheimer's disease. Neuroscience research 2006; 54(3): 197-201.
Mudher A, Colin M, Dujardin S, Medina M, Dewachter I, Alavi Naini SM*, et al.* What is the evidence that tau pathology spreads through prion-like propagation? Acta neuropathologica communications 2017; 5(1): 99.
Oroz J, Chang BJ, Wysoczanski P, Lee CT, Perez-Lara A, Chakraborty P*, et al.* Structure and pro-toxic mechanism of the human Hsp90/PPIase/Tau complex. Nat Commun 2018; 9(1): 4532.
Ozcelik S, Fraser G, Castets P, Schaeffer V, Skachokova Z, Breu K*, et al.* Rapamycin Attenuates the Progression of Tau Pathology in P301S Tau Transgenic Mice. PLoS One 2013; 8(5): e62459.
Ozcelik S, Sprenger F, Skachokova Z, Fraser G, Abramowski D, Clavaguera F*, et al.* Co-expression of truncated and full-length tau induces severe neurotoxicity. Mol Psychiatry 2016; 21(12): 1790-8.
Patterson KR, Remmers C, Fu Y, Brooker S, Kanaan NM, Vana L*, et al.* Characterization of prefibrillar Tau oligomers in vitro and in Alzheimer disease. J Biol Chem 2011; 286(26): 23063-76.
Polanco JC, Li C, Bodea LG, Martinez-Marmol R, Meunier FA, Gotz J. Amyloid-beta and tau complexity - towards improved biomarkers and targeted therapies. Nature reviews Neurology 2018; 14(1): 22-39.
Polydoro M, de Calignon A, Suarez-Calvet M, Sanchez L, Kay KR, Nicholls SB*, et al.* Reversal of neurofibrillary tangles and tau-associated phenotype in the rTgTauEC model of early Alzheimer's disease. J Neurosci 2013; 33(33): 13300-11.
Probst A, Gotz J, Wiederhold KH, Tolnay M, Mistl C, Jaton AL*, et al.* Axonopathy and amyotrophy in mice transgenic for human four-repeat tau protein. Acta neuropathologica 2000; 99(5): 469-81.
Puangmalai N, Bhatt N, Montalbano M, Sengupta U, Gaikwad S, Ventura F*, et al.* Internalization mechanisms of brain-derived tau oligomers from patients with Alzheimer's disease, progressive supranuclear palsy and dementia with Lewy bodies. Cell Death Dis 2020; 11(5): 314.
Sanders DW, Kaufman SK, DeVos SL, Sharma AM, Mirbaha H, Li A*, et al.* Distinct tau prion strains propagate in cells and mice and define different tauopathies. Neuron 2014; 82(6): 1271-88.

Santacruz K, Lewis J, Spires T, Paulson J, Kotilinek L, Ingelsson M*, et al.* Tau suppression in a neurodegenerative mouse model improves memory function. Science 2005; 309(5733): 476-81.
Schaeffer V, Lavenir I, Ozcelik S, Tolnay M, Winkler DT, Goedert M. Stimulation of autophagy reduces neurodegeneration in a mouse model of human tauopathy. Brain 2012; 135(Pt 7): 2169-77.
Sekiya H, Kowa H, Koga H, Takata M, Satake W, Futamura N*, et al.* Wide distribution of alpha-synuclein oligomers in multiple system atrophy brain detected by proximity ligation. Acta neuropathologica 2019; 137(3): 455-66.
Sengupta U, Montalbano M, McAllen S, Minuesa G, Kharas M, Kayed R. Formation of Toxic Oligomeric Assemblies of RNA-binding Protein: Musashi in Alzheimer's disease. Acta neuropathologica communications 2018; 6(1): 113.
Soeda Y, Yoshikawa M, Almeida OF, Sumioka A, Maeda S, Osada H*, et al.* Toxic tau oligomer formation blocked by capping of cysteine residues with 1,2-dihydroxybenzene groups. Nat Commun 2015; 6: 10216.
Spillantini MG, Goedert M. Tau pathology and neurodegeneration. Lancet Neurol 2013; 12(6): 609-22.
Spires-Jones TL, Hyman BT. The intersection of amyloid beta and tau at synapses in Alzheimer's disease. Neuron 2014; 82(4): 756-71.
Van der Jeugd A, Hochgrafe K, Ahmed T, Decker JM, Sydow A, Hofmann A*, et al.* Cognitive defects are reversible in inducible mice expressing pro-aggregant full-length human Tau. Acta neuropathologica 2012; 123(6): 787-805.
Wang X, Smith K, Pearson M, Hughes A, Cosden ML, Marcus J*, et al.* Early intervention of tau pathology prevents behavioral changes in the rTg4510 mouse model of tauopathy. PLoS One 2018; 13(4): e0195486.

Figures

Figure1

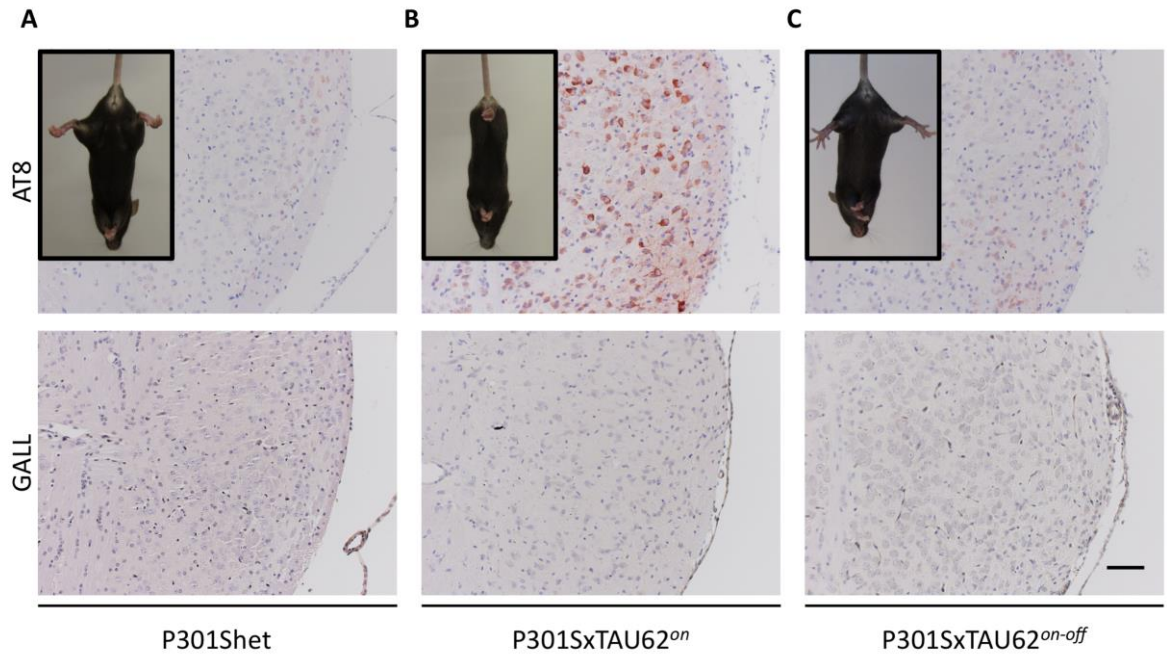


Figure 1: Early neurotoxic stress depends on full length and Δtau co-expression and is reversible. P301S heterozygous mice (P301Shet; n=5) at 21 days of age show no signs of paralysis in the tail suspension test, and histological tests with AT8 antibody did not detect hyperphosphorylated tau or Gallyas-positive tau fibrils in the tegmental reticular nucleus (A). Hindlimbs of P301SxTAU62^{on} (n=5) mice the same age as their heterozygous littermates are paralyzed; upon histological characterization, paralyzed P301SxTAU62^{on} mice show hyperphosphorylated tau but no Gallyas-positive tau fibrils in the same region (B). After suspending doxycycline administration for 3 weeks, P301SxTAU62$^{on\text{-}off}$ (n=5) mice recover their motor functions and do not show hyperphosphorylated or fibrillar tau (C). Altogether, the findings confirm that the severe neurotoxicity in P301SxTAU62^{on} mice does not depend on filamentous tau pathology, but solely on hyperphosphorylated tau oligomers. Scale bar equals 75 µm, and applies to A, B and C.

Figure 2

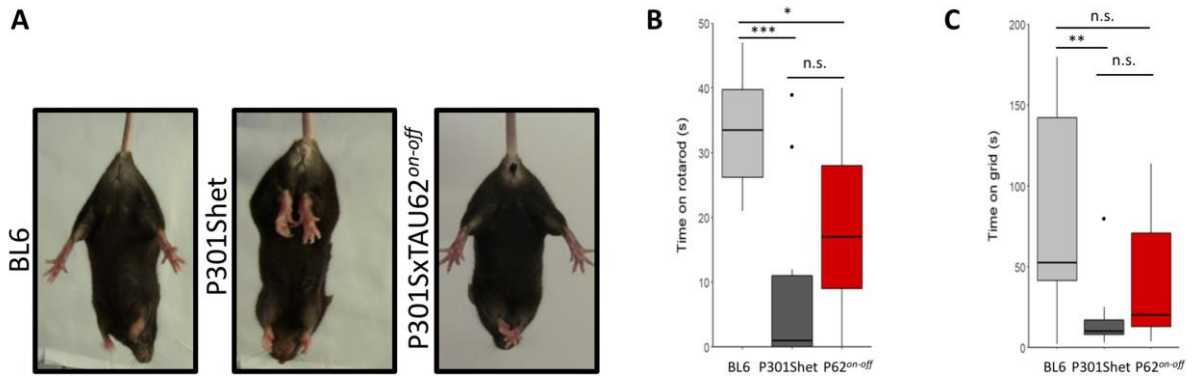


Figure 2: P301SxTAU62$^{on\text{-}off}$ mice do not develop an impaired motor phenotype upon aging.

Tail suspension tests revealed normal hindlimb spreading for BL6 mice, and pathological hindlimb spreading predominantly in heterozygous P301S transgenic littermates at 16 months of age, while this was less pronounced in age-matched P301SxTAU62$^{on\text{-}off}$ (abbreviated P62$^{on\text{-}off}$) mice (A). Rotarod test comparing BL6 (n=10), P301Shet (n=15), and P301SxTAU62$^{on\text{-}off}$ mice (n=13) at 16 months of age revealed that P301SxTAU62$^{on\text{-}off}$ animals were not significantly more impaired than P301Shet mice which did not undergo early neurotoxic stress (B, P-value = 0,14). Grid tests of BL6 (n=10), P301Shet (n=17), and P301SxTAU62$^{on\text{-}off}$ mice (n=11) at 16 months also showed that P301SxTAU62$^{on\text{-}off}$ mice were not experiencing heavier motor impairment when compared to their heterozygous littermates (C, P-value= 0,09). n.s. = P-value > 0,05; * = P-value < 0,05; ** = P-value < 0,01; *** = P-value < 0,001. See also Supplementary Table 1.

Figure 3

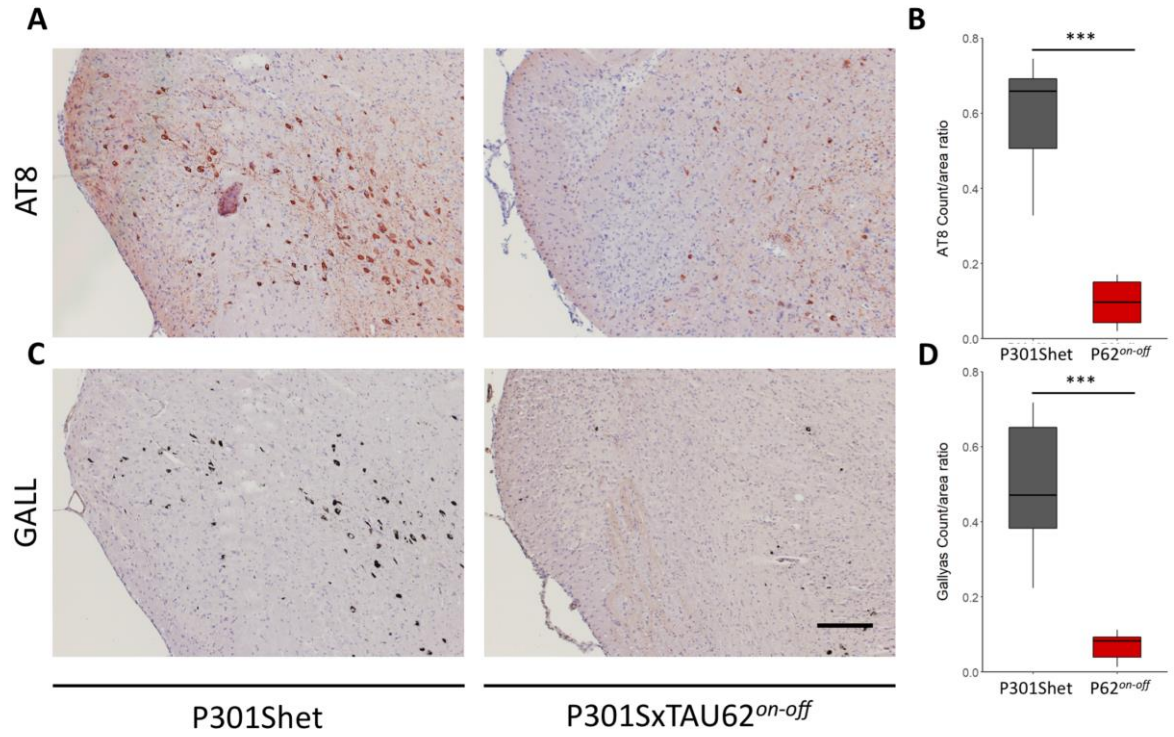


Figure 3: Aged P301SxTAU62*on-off* mice show decreased hyperphosphorylated and fibrillar tau pathology compared to their heterozygous littermates.

Histological tests comparing AT8 stained brainstem sections (A) of 16-months-old P301Shet (n=7) and P301SxTAU62*on-off* (abbreviated P62*on-off*) mice (n=7) revealed that the different performance in the behavioral tests was paralleled by different degrees of hyperphoshorylated tau pathology (B, P-value = 3,39E-06). These results were also mirrored by Gallyas-stained brainstem sections (C) of 16-months-old P301Shet (n=7), and P301SxTAU62*on-off* mice (n=7), which revealed different degrees of fibrillar tau pathology between the two groups (D, P-value = 7,77E-05). *** = P-value < 0,001. Scale bar equals 200 µm, and applies to A and C. See also Supplementary Table 2.

Figure 4

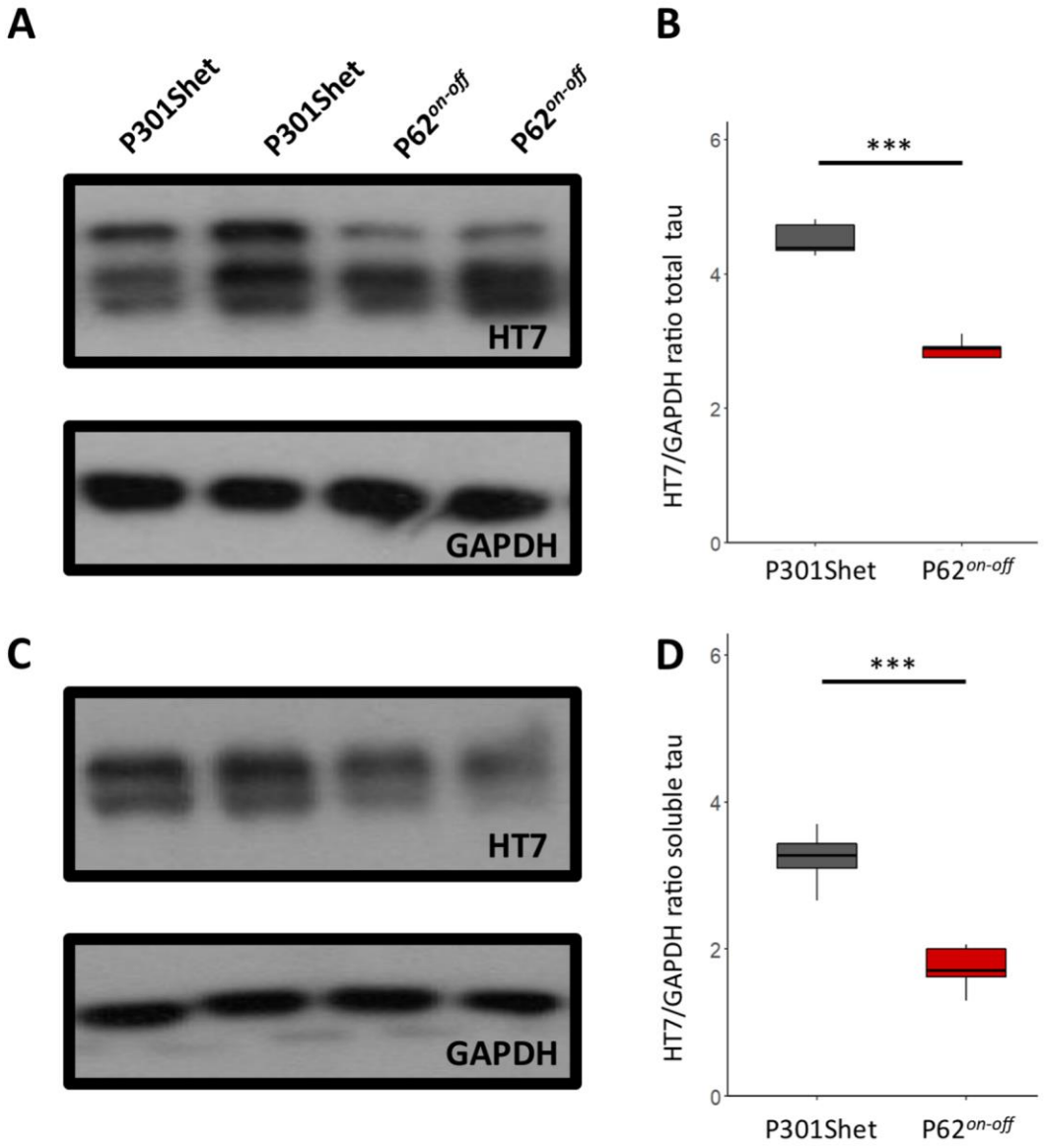


Figure 4: Aged P301SxTAU62^{off} mice show lower total and soluble tau levels than their heterozygous littermates.

Western blot analysis of total tau (HT7 antibody) revealed that P301Shet mice (n=5) have a significantly higher level of total tau when compared to P301SxTAU62$^{on\text{-}off}$ (abbreviated P62$^{on\text{-}off}$) mice (n=5) at 16 months of age (A), which was confirmed by HT7/GAPDH ratio quantification (B, P-value = 1,32E-06). Soluble tau levels (C) in P301Shet mice (n=5) were also significantly higher than in P301SxTAU62$^{on\text{-}off}$ mice (n=5), as reflected by HT7/GAPDH ratio quantification (D, P-value = 0,0001). *** = P-value < 0,001. See also Supplementary Table 2.

Figure 5

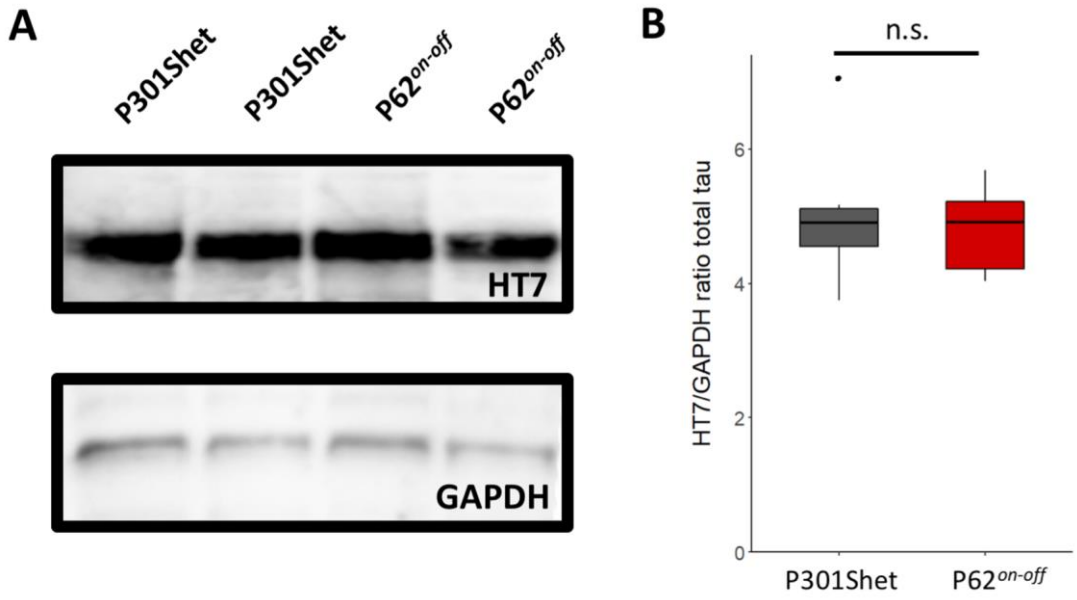


Figure 5: Total tau levels in young P301SxTAU62$^{on\text{-}off}$ mice do not decrease compared to their heterozygous littermates.

Western blot analysis (HT7; A) and HT7/GAPDH ratio (B) for both P301Shet (n=6) and P301SxTAU62$^{on\text{-}off}$ (abbreviated P62$^{on\text{-}off}$) mice (n=6). Tau levels were not significantly different between the two groups (P-value = 0,67). n.s. = P-value > 0,05. Box plots with hinges, whiskers and outliers. See also Supplementary Table 2.

Figure 6

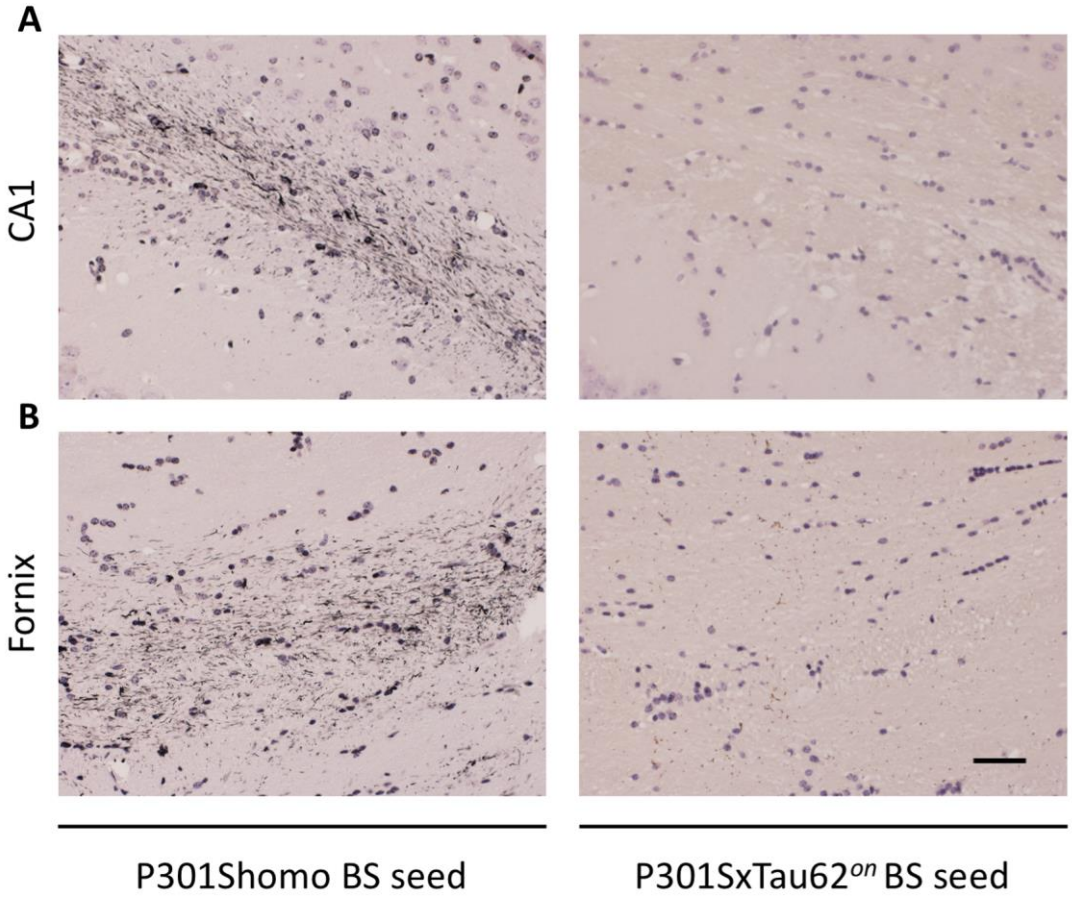
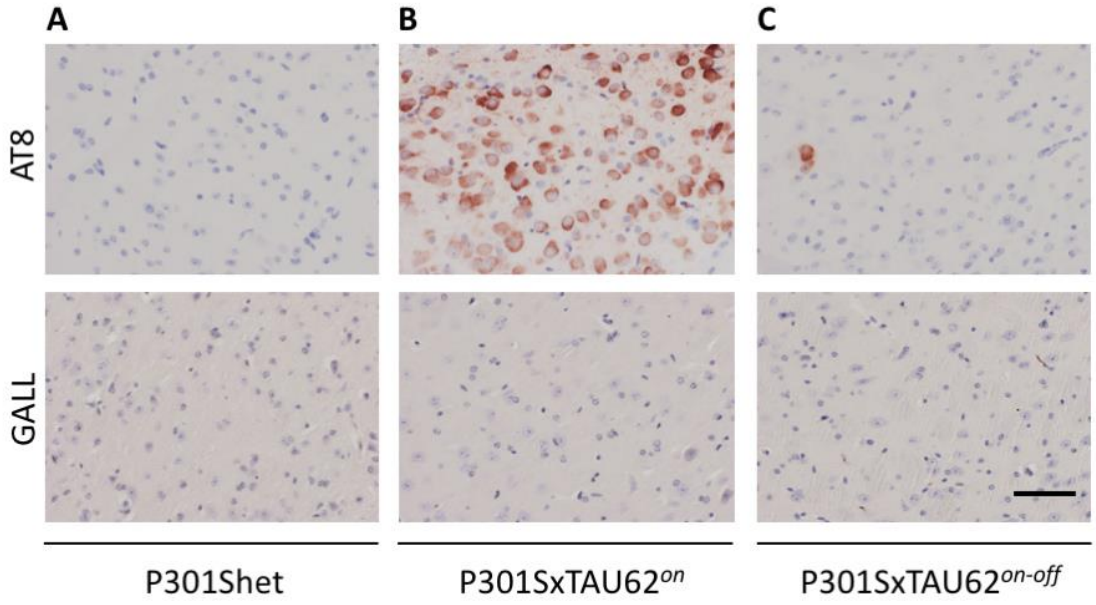


Figure 6: Seeding with high molecular weight tau results in complete absence of fibrils in ALZ17 mice.

ALZ17 mice seeded with brainstem homogenates from paralyzed P301S homozygous (abbreviated P301Shomo) mice (n=5) revealed granular focal tau pathology in CA1 (A), which was not detected in ALZ17 mice seeded with P301SxTAU62^{on} brainstem homogenate (n=4). Granular focal tau pathology was also found in the fornix (B), and Gallyas-positive structures were detected in ALZ17 mice seeded with brainstem homogenates of paralyzed P301Shomo mice, but not in ALZ17 mice seeded with P301SxTAU62^{on} brainstem homogenate. Scale bar equals 37,5 µm, and applies to A and B.

2.2.1 Supplementary Material

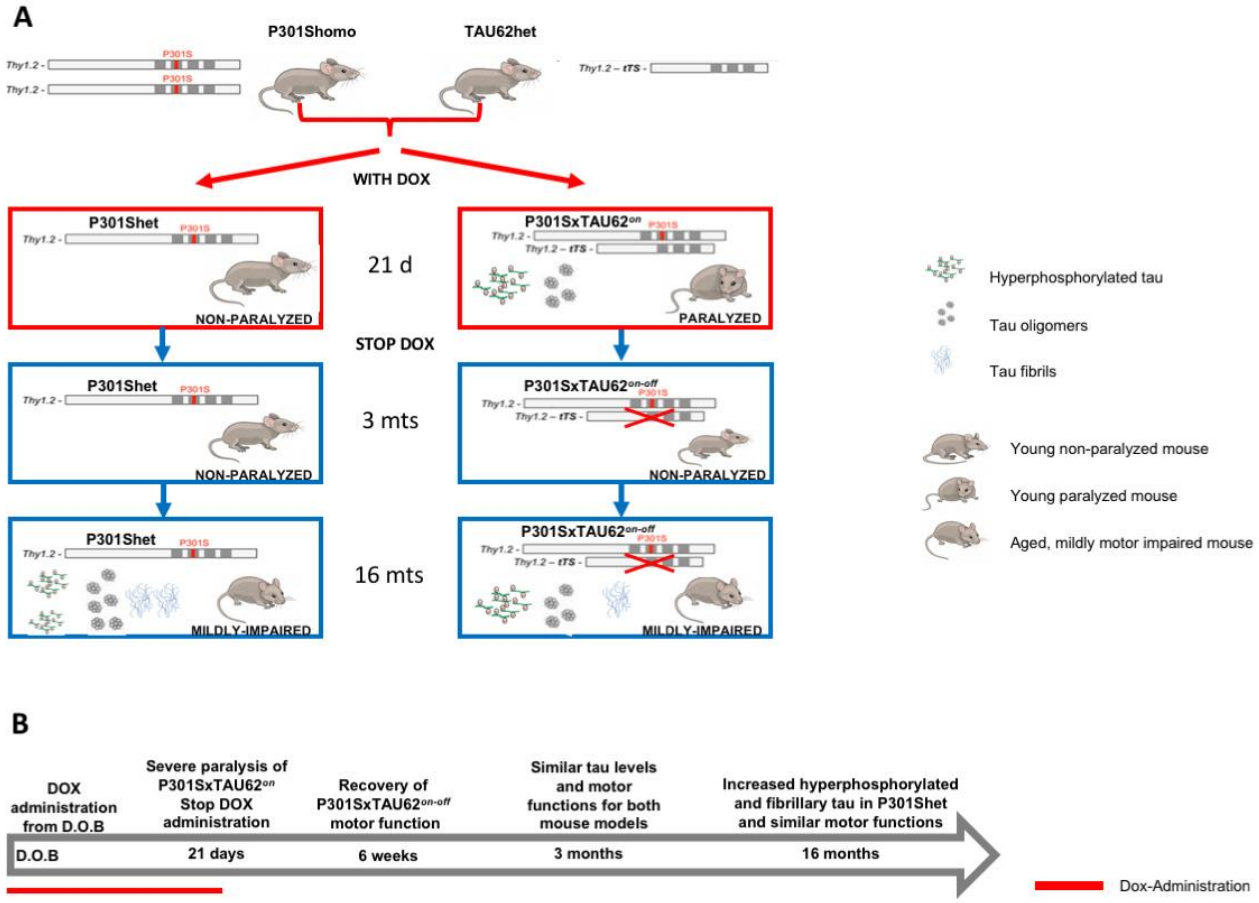
Supplementary Figure 1:



Suppl. Fig. 1: High magnification images extending the findings shown in Figure 1.

P301S heterozygous mice (P301Shet; n=5) at 21 days of age show no signs of hyperphosphorylated tau or Gallyas-positive tau fibrils in the tegmental reticular nucleus (A). Paralyzed P301SxTAU62^{on} mice (n=5) show hyperphosphorylated tau but no Gallyas-positive tau fibrils in the same region (B). After suspending doxycycline administration for 3 weeks, P301SxTAU62$^{on\text{-}off}$ mice (n=5) do not show hyperphosphorylated or fibrillar tau (C). Scale bar equals 50 µm, and applies to A, B and C.

Supplementary Figure 2:

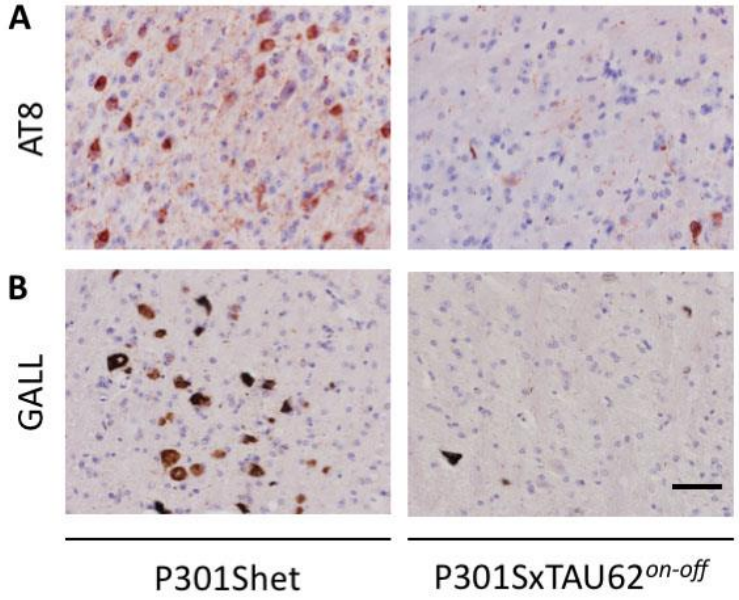


Suppl. Fig. 2: Schematic of background and main conclusions showing no long-term sequelae of reverted tau toxicity.

P301SxTAU62^{on} mice co-express human P301S mutant tau and, only under oral doxycycline (DOX) administration, $\Delta tau_{151\text{-}421}$ fragment. This results in hyperphosphorylated tau oligomers that cause a severe paralysis after 21 days. If these mice are fed normally, they only express heterozygous mutant P301S tau but not the tau fragment, and are thus comparable to P301Shet mice which remain without oligomeric tau formation or severe paralysis after 21 days. After expression of the doxycycline-responsive $\Delta tau_{151\text{-}421}$ fragment is switched off (P301SxTAU62$^{on\text{-}off}$), hyperphosphorylated tau oligomers are cleared efficiently and mice recover their motor function in the following 3 weeks, even though mutant P301S tau expression is being maintained. Therefore, P301SxTAU62$^{on\text{-}off}$ mice exhibit a similar phenotype as P301Shet mice at three months of age. At 16 months of age, P301SxTAU62$^{on\text{-}off}$ mouse motor functions do not differ significantly from P301Shet mice, while tangles and hyperphosphorylated

tau oligomers are significantly less abundant in P301SxTAU62$^{on\text{-}off}$ compared to P301Shet mice. This proves that P301SxTAU62$^{on\text{-}off}$ mice do not experience sequelae of the early neurotoxic stress, when compared to their heterozygous P301S tau transgenic littermates.

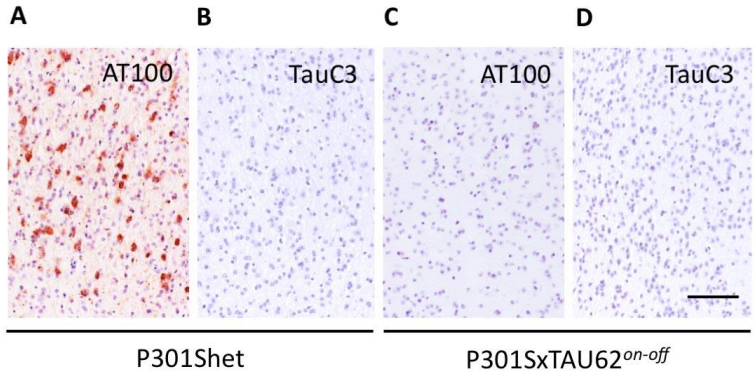
Supplementary Figure 3:



Suppl. Fig. 3: High magnification images extending the findings shown in Figure 3.

Aged P301SxTAU62$^{on\text{-}off}$ mice exhibit less AT8 and Gallyas-positive neurons than their heterozygous littermates. AT8 (A) and Gallyas silver (B) stained brainstem sections of 16-months-old P301Shet mice (n=7) show more hyperphosphorylated tau and silver stain positive pathology than P301SxTAU62$^{on\text{-}off}$ mice (n=7). Scale bar equals 50 µm, and applies to A and B.

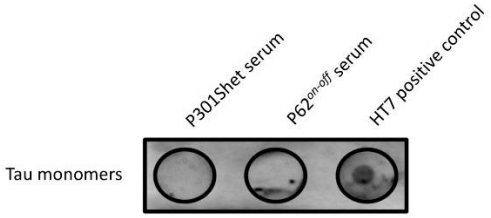
Supplementary Figure 4:



Suppl. Fig. 4: Immunohistochemistry targeting the AT100 phospho-epitopes at Thr 212 and Ser 214, and Δtau (TauC3 antibody) in 16-months-old mice.

In parallel to Gallyas silver stain positivity (Suppl. Fig. 3), extensive hyperphosphorylation of AT100-positive tau was seen in the brainstem of heterozygous P301S littermates (n=7) (A), but was almost absent in P301SxTAU62$^{on\text{-}off}$ mice (n=7) (C). Immunohistochemistry with TauC3 antibody did not find any tau fragment in non-fragment expressing P301Shet mice (n=7) (B), nor were signs of fragment expression leakage detectable in P301SxTAU62$^{on\text{-}off}$ mice (n=7) (D). The scale bar corresponds to 100 µm, and applies from A to D.

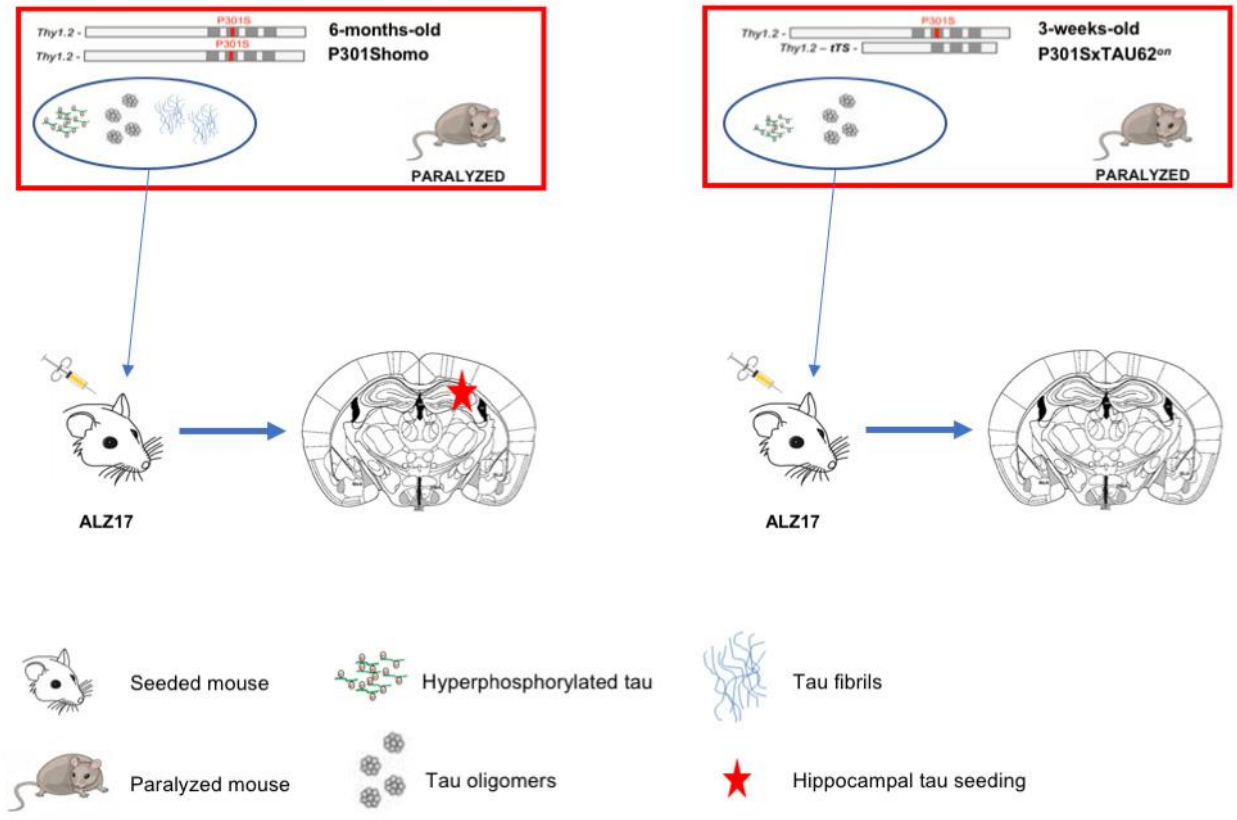
Supplementary Figure 5:



Suppl. Fig. 5: 3-months-old P301Shet and P301SxTAU62$^{on\text{-}off}$ mice do not show anti-tau antibodies in their sera.

Dot blot with the sera of 3-months-old P301Shet (n=4) and P301SxTAU62$^{on\text{-}off}$ (labelled P62 $^{on\text{-}off}$; n=3) mice was conducted, to test for the presence of anti-tau antibodies, using HT7 antibody as a positive control. 2N4R wild-type tau monomers were applied on the membrane and both sera did not show any immunological activity.

Supplementary Figure 6:



Suppl. Fig. 6: Schematic of background and main conclusions showing that oligomeric toxic tau species can lack seeding competence.

As positive control, ALZ17 mice were injected with tau fibril-containing brainstem homogenates of paralyzed, 6-months-old P301S mice (left box). These ALZ17 control mice developed granular focal tau pathology in the injected hippocampus. Next, we analyzed the seeding capacity of oligomers resulting from co-expression of P301S tau and $\Delta tau_{151\text{-}421}$. To this end, we inoculated ALZ17 transgenic mice with brainstem homogenates collected from paralyzed, 3-weeks-old P301SxTAU62^{on} mice, which do not show fibrillary tau pathology (right box). ALZ17 mice seeded with P301SxTAU62^{on} mice brainstem homogenates did not develop focal tau aggregates in the inoculated hippocampus. This proves that brainstem tissue of paralyzed P301SxTAU62^{on} mice lacks *in vivo* tau seeding competence.

Supplementary Table 1:

TEST	***Mean time ± STD BL6***	***Mean time ± STD P62^{on-off}***	***Mean time ± STD P301Shet***	***P-value ANOVA***	***P-value BL6 vs P301Shet***	***P-value BL6 vs P62^{on-off}***	***P-value P301Shet vs P62^{on-off}***
Rotarod 16 months	33 s ± 8,77 s	17,46 s ± 12,78 s	7,73 s ± 12,01 s	2,8E-05 (***)	2,5E-05 (***)	0,01 (*)	0,14 (n.s)
Grid 16 months	81,9 s ± 65,14 s	40,27 s ± 38,84 s	15,35 s ± 17,58 s	0.001 (**)	0,001 (**)	0,4 (n.s)	0.09 (n.s)

Suppl. Table 1: Overview on behavioral tests.

The table shows the overview of the statistical values of the behavioral tests executed on P301S heterozygous mice (P301Shet), P301SxTAU62^{on-off} mice (P62^{on-off}) and BL6 mice; n.s = P-value > 0,05; * = P-value < 0,05; ** = P-value < 0,01; *** = P-value < 0,001.

Supplementary Table 2:

TEST	***Mean ± STD P301Shet***	***Mean ± STD P62^{on-off}***	***P-value P301Shet vs P62^{on-off}***
AT8 Count/area 16 months	0,59 ± 0,15	0,10 ± 0,06	3,39E-06 (***)
Gallyas Count/area 16 months	0,50 ± 0,19	0,07 ± 0,04	7,77E-05 (***)
HT7/GAPDH signal total tau WB 16 months	4,51 ± 0,24	2,88 ± 0,15	1,32E-06 (***)
HT7/GAPDH signal soluble tau WB 16 months	3,23 ± 0,39	1,71 ± 0,31	0,0001 (***)
HT7/GAPDH signal WB 3 months	5,03 ± 1,11	4,81 ± 0,67	0,67 (n.s)

Suppl. Table 2: Overview on immunohistochemistry and western blots.

The table shows the overview of the statistical values of the quantified stained sections and western blots on 3 and 16 months old P301S heterozygous (P301Shet) mice and P301SxTAU62^{on-off} (P62^{on-off}) mice; n.s = P-value > 0,05; * = P-value < 0,05; ** = P-value < 0,01; *** = P-value < 0,001.

3 Supplementary Results

3.1 Sup35NM is able to promote the vascular aggregation of amyloid-beta (Aβ)

After the promising results of our study “Cross-seeding of Alzheimer-related prion-like proteins by non-mammalian prions” where we investigated the potential of Sup35NM fibrils to accelerate the aggregation of tau *in vitro* and *in vivo*, we decided to also investigate the potential of Sup35NM fibrils to accelerate the formation of amyloid-beta (Aβ) aggregates. Strikingly in a first trial the inoculation of App23 mice, expressing human amyloid precursor protein (APP) bearing the pathogenic Swedish mutation (Sturchler-Pierrat *et al.*, 1997), led to the accelerated formation of vascular Aβ aggregates in the hippocampus of the mice specific for the brain half of the Sup35NM inoculation. The results are shown below in figure 4. The difference between injected and non-injected hippocampal side was stronger after an increased time of incubation. While after 14 months of incubation the difference in Aβ deposition was mainly quantitative (fig. 4b) after 19 months of incubation the affected vessels were mostly filled with Aβ aggregates on the injected brain halve while on the non-injected brain halve the deposition of Aβ was mainly limited to the vessel walls (fig. 4a). Compared to seeding with Sup35NM, seeding of App23 mice with -5TyrSup35NM monomers only led to a very limited increase in Aβ aggregation (fig. 4c). This is in coherence with our results for the seeding potential of Sup35NM and -5TyrSup35NM concerning tau aggregation where -5TyrSup35NM also only showed a mild residual tau aggregation induction capacity. This is further evidence, that structural similarities, in this case the presence of an in-register beta sheet structure in Sup35NM, Aβ and tau aggregates, are necessary for an efficient prion seeding effect. This is also in line with recent scientific results by others, showing that aggregates, but not monomers, of Aβ, tau, and α-synuclein bind to PrP (Corbett *et al.*, 2019).

Altogether these preliminary results about Sup35NM and its ability to seed Aβ aggregation support our hypothesis of a structure based prion seeding of prion-like proteins like tau and Aβ as a potential way on how a natural prion from the human microbiome like Sup35 can influence the development and progression of neurodegenerative diseases like AD.

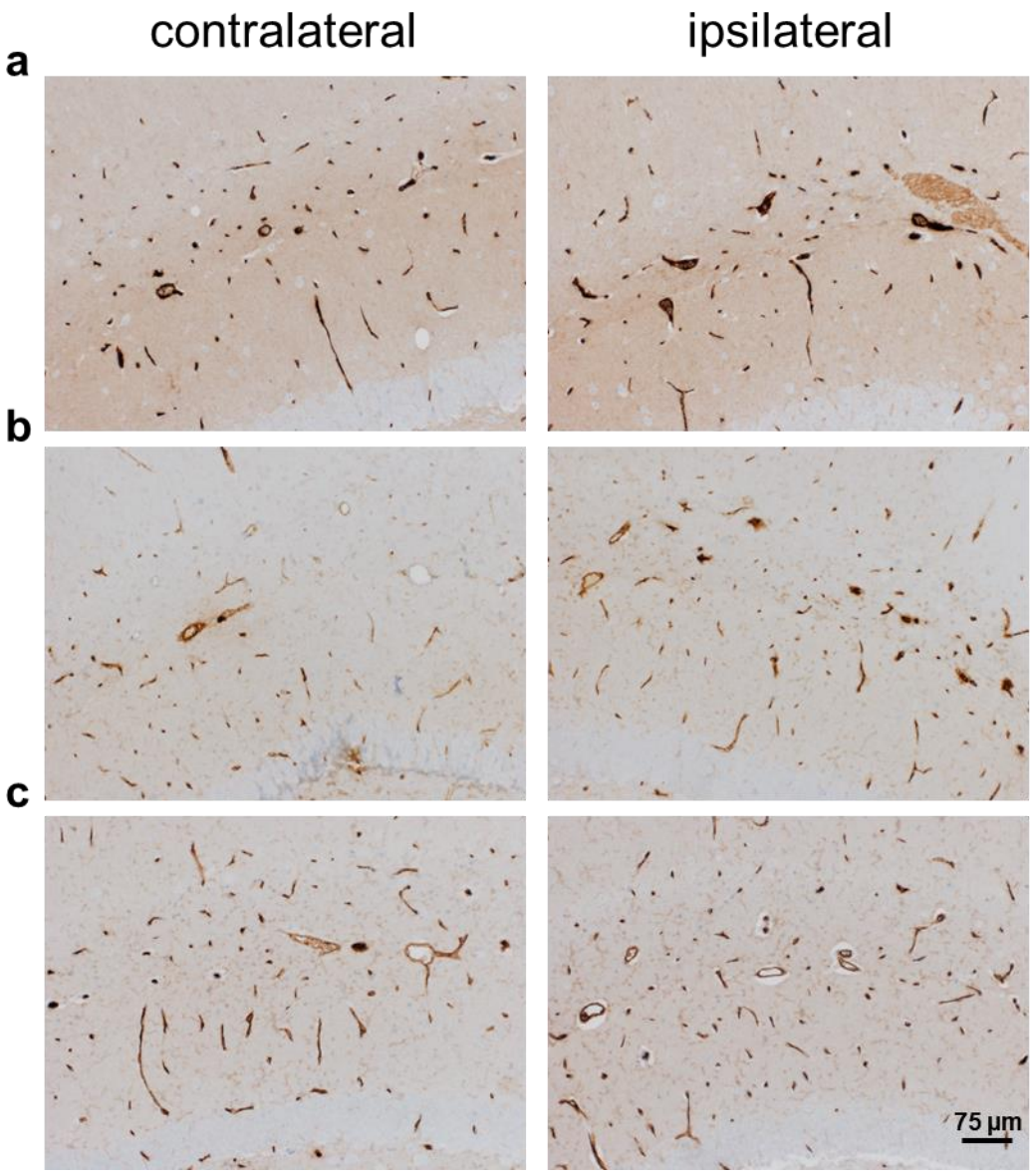


Figure 4: Sup35 seeds Aβ aggregation in App23 mice

a-c, increase in immunohistochemistry (Anti Aβ antibody; Agilent, Cat# M0872) positive vascular Aβ deposits in the hippocampal CA1 field following unilateral intrahippocampal Sup35NM fibril inoculation (a-b) or -5TyrSup35NM monomer inoculation (c) in App23 Aβ transgenic mice. Comparison of the non-injected side (contralateral) to the injected side (ipsilateral) in representative histological findings (Bregma level -2.4mm) after 19 months of incubation with Sup35NM (a), 14 months of incubation with Sup35NM (b) or 15 months of incubation with -5TyrSup35NM (c).

3.2 Modulating tau toxicity by Cabazitaxel

Cabazitaxel is a taxane which was shown to be able to pass the blood brain barrier (BBB) two- to three-fold better than the taxanes docetaxel and paclitaxel. Additionally, Cabazitaxel is distributed well throughout the brain parenchyma and has a way longer retention time in the mouse brain than in the mouse blood (Cisternino *et al.*, 2003; Girard *et al.*, 2015). This makes it an ideal candidate drug for the approach to stabilize microtubules in the brain while minimizing the likelihood of side effects. In AD tau gets hyperphosphorylated and leaves the microtubules, which leads to microtubule destabilization and therefore to a disturbance of axonal transport (Kadavath *et al.*, 2015). In accordance with this the taxane epothilone D (EpoD) was able to reduce axonal dysfunction in the PS19 mouse model of tauopathy (Zhang *et al.*, 2012). However, a clinical Phase 1 trial to evaluate the tolerability and pharmacology of EpoD was discontinued (ClinicalTrials.gov Identifier: NCT01492374). P301SxTAU62on mice exhibit extensive signs of axonal transport disruption (Ozcelik et al 2016). This renders them particularly suitable for the analysis whether microtubule stabilizing agents can rescue tau oligomer induced axonal transport failure.

Additionally, due to the rapid onset of motor impairments in P301SxTAU62on mice, significant behavioral responses can be measurable already within 3 weeks after initiation of treatment. Against this background, we assessed the microtubule stabilizer Cabazitaxel.

In order to first examine potential side effects of Cabazitaxel we started the treatment with a low dose of 1mg/kg. Cabazitaxel was solved in a solution consisting of 5% ethanol, 5% polysorbate and 90% aqueous solution containing 5% sucrose. This is the same application vehicle which is used in the treatment of human cancer patients with Cabazitaxel (Lazzarini *et al.*, 2015). For the first trial 3 mice were injected with Cabazitaxel, 2 mice with vehicle only and 2 mice served as uninjected controls. The mice were injected once a week for three weeks. With these doses there were no visible side effects for mice injected with Cabazitaxel compared to mice, with the same genotype, which were injected only with the vehicle or not injected at all. However, unfortunately the treatment with Cabazitaxel also did not result in any phenotypical slowing of the developing paresis. Therefore, and for the absence of side effects, we decided to increase the dosage of Cabazitaxel to 2mg/kg bodyweight. For this second trial 6 mice were injected with Cabazitaxel, 6 mice with vehicle only and 3 mice served as uninjected controls. While again not seeing any side effects there was again no visible deceleration of paresis compared to control mice.

As the clinical trial to evaluate the tolerability and pharmacology of EpoD in humans was stopped with doses between 0.01 mg/kg and 0.003 mg/kg, we considered that the risk of harming the test mice by further increasing the dosage of Cabazitaxel would outweigh the chance of potential benefits compared to control mice. Therefore, we decided to discontinue our trials with Cabazitaxel in order to adhere to the 3R principles of animal ethics.

After our decision to discontinue our experiments with Cabazitaxel the results of a Phase 1 trial to evaluate the safety, tolerability, pharmacokinetics, pharmacodynamics and preliminary efficacy of Cabazitaxel (taxane TPI-287) in AD patients were published (Tsai *et al.*, 2019). This trial had been completed in November 2019 (ClinicalTrials.gov Identifier: NCT01966666). In this study treatment with TPI-287 led to severe anaphylactoid reactions, more falls and a dose-related worsening of dementia symptoms (Tsai et al., 2019). These results show that our decision to discontinue our trials with Cabazitaxel was wise.

4 Material and methods

4.1 Mice

Homozygous human P301S mutant tau transgenic mice (P301S tau mice) (Allen *et al.*, 2002) were used as host mice for tau seeding experiments with Sup35NM. Heterozygous App23 mice overexpressing mutant human amyloid precursor protein (APP) bearing the pathogenic Swedish mutation (Sturchler-Pierrat et al., 1997) were used for amyloid beta (Aβ) seeding experiments with Sup35NM. Transgenic homozygous mice expressing human ALZ17 mutant tau (ALZ17 mice) (Probst *et al.*, 2000) were used as host mice for tau seeding experiments with brain stem homogenates from P301SxTAU62 mice. P301SxTAU62 mice were obtained by interbreeding P301S mice and TAU62 mice (Ozcelik *et al.*, 2016). TAU62 mice express a human tau fragment from amino acid 151-421 (Δtau) (Ozcelik *et al.*, 2016). P301SxTAU62 mice were used for the investigation of potential long-term effects of neurotoxicity caused by tau oligomers, and for the Cabazitaxel trials. The mice were housed with a 12-h light/dark cycle and permanent access to food and water. Animal experiments were approved by the official local Committee for Animal Care and Animal Use of the Canton of Basel (Licenses Nr. BS 2364 and 2471).

4.2 Stereotaxic surgery

Three months-old P301S tau and App23 mice were anaesthetized with a mixture of ketamine (10 mg/kg) and xylazine (20 mg/kg) and placed on a heating pad to maintain body temperature during surgery. Mice were injected in the right hippocampus (A/P, −2.5 mm from bregma; L, −2.0 mm; D/V, −1.8 mm) using a Hamilton syringe. Each mouse received a unilateral stereotaxic injection of 2.5 µl volume, at a speed of 1.25 µl/min. Following the injection, the needle was kept in place for an additional 3 min. The surgical area was cleaned with saline and the incision sutured. Mice were monitored until recovery from anesthesia, provided analgesic medication post-surgery, and checked regularly following surgery. Alz17 were operated under the same conditions however the injected volume was 5 µl for these mice.

4.3 Tissue homogenization

Brainstem homogenates of 6-months old paralyzed homozygous P301S mice, 3-weeks old paralyzed P301SxTAU62on mice and C57BL/6 mice (B6 mice) were prepared for later inoculation in P301S, App23 or ALZ17 mice.
Brainstems were weighted and diluted 1:9 in PBS for seeding and TBS-Complete for Western blots. For both procedures, after dilution, the samples were homogenized using an Ultraturrax T8 (IKA labortechnik, Staufen im Breisgau, Germany) and briefly sonicated (Bandelin SONOPULS, 90% power, 10% cycle, 10 sec pulses). The homogenates were then centrifuged at 4000 g for 20 minutes, at 4 °C, and the aliquots of the resulting supernatant were stored at -80 °C for later usage.

4.4 Expression, purification and fibrillization of Sup35NM

The combined N-terminal (N) and middle (M) domains of Sup35 are sufficient to form Sup35NM fibrils harboring prion properties (Sparrer *et al.*, 2000). The Sup35NM expression plasmid p1404 was a kind gift from Prof. Reed B. Wickner (Laboratory of Biochemistry and Genetics, NIH, Bethesda, U.S.A.). *E. coli* Rosetta (DE3) pLysS was used as expression host. The transformation of the *E. coli* was performed using the heat shock method (Froger and Hall, 2007). Transfected cells were selected using LB plates containing Ampicillin (50 µg/ml) and Kanamycin (30 µl/ml). A transformed colony was incubated overnight at 37 °C in a 50 ml LB-ampicillin-kanamycin flask. On the next day 20 ml of growing culture were added to a fresh flask containing 2L TB-ampicillin-kanamycin medium.

Induction was performed with 1 mM Isopropyl β-d-1-thiogalactopyranoside (IPTG) at an optical absorption at 600 nm wavelength (A_{600}) of approx. 1. The cells were harvested after 5h of IPTG induction. The cell pellets were collected by centrifugation and lysed by sonication in lysis buffer (50 mM Tris-HCL, 1 mM phenylmethylsulfonyl fluoride (PMSF), 8 M urea, pH 8.0).

The lysate was cleared using centrifugation at 12,000 g for 20 min at 4 °C. The cleared supernatant was applied to a 1 ml HisTrap™ (GE Healthcare, Chicago, US) column pre-equilibrated with lysis buffer. The column was washed with wash buffer (50 mM Tris-HCl, 1 mM DTT, 8 M urea, 300 mM NaCl and 20 mM imidazole) until the absorption at 280 nm reached zero. The target protein was eluted using 250 mM imidazole. The protein in each fraction was monitored using a spectrophotometer. The purity of Sup35NM in the selected fractions was analyzed by 4-12% SDS PAGE.

The protein was then loaded onto a HiLoad 16/60 Superdex 75 PG (GE Healthcare, Chicago, US) column equilibrated with 7 M urea, 50 mM Tris (pH 7.5) and 150 mM NaCl.

The fractions containing Sup35NM were assessed via WB using an anti His-Tag antibody (Anti-6X His tag® antibody (HRP), Abcam, Cambridge, UK), pooled, concentrated and dialyzed against PBS using a Slide-A-Lyzer 10K dialysis cassette (Thermo Fisher Scientific, Waltham, US). Sup35NM was then concentrated to 0.271 mg/ml using an Amicon ultra-centrifugal filter with a 10K cutoff (Millipore, Burlington, US). Afterwards, Sup35NM was placed in a cold room at 4 °C under slight agitation for 3 days in order to enable fibrillization into the desired conformation (Ohhashi *et al.*, 2010). Aliquots of fibrilized samples were stored at -80 °C prior to the inoculations.

4.5 Preparation of -5TyrSup35NM and Cabazitaxel

-5TyrSup35NM was obtained from GenScript (Piscataway, US), diluted in PBS solvent to a concentration of 0.3 mg/ml and stored at -80 °C prior to the inoculation. Cabazitaxel was obtained from Lucerna-Chem AG (Luzern, CH) and solved in a solution consisting of 5% ethanol, 5% polysorbate and 90% aqueous solution containing 5% sucrose.

4.6 Behavioral tests

Motor behavior, including gait ataxia, tremor and hind limb reflexes, was assessed. Quantitative motor testing was performed by the grid test in which mice were placed on a vertical mesh grid and the latency to fall off from the grid was recorded for 3 min. The motor coordination and balance of mice were assessed using the Panlab Harvard Rotarod (Panlab, Barcelona, ES). The Rotarod starts at a speed of 4 rpm and accelerates consistently with 1 rpm every 3 sec. For both tests, the mice were tested for 3 consecutive days with 3 trials each day and a rest interval of 5 min minimum, and the mean latency to fall was documented. Results were obtained by averaging the daily means of 3 consecutive days, and the daily means were obtained by 3 trials per day.

4.7 Sacrificing of mice and tissue preparation

Mice were deeply anaesthetized with a mixture of 2% Rompun (5 mg/kg) / 10% Ketamin (100 mg/kg) 0.2 ml / 10 g body weight i.p. using a 25G hypodermic needle. Once in deep narcosis and no longer responding to stimuli, the mice were transcardially perfused with 20 ml cold PBS, followed by 20 ml 4% paraformaldehyde in PBS. The brains were dissected and post-fixed overnight. Following paraffin embedding, 4 µm coronal sections were cut from the brain tissues of seeded mice, while 4 µm sagittal sections were cut from the brain tissues of the mice used for the behavioral tests. For Western blots and seeding material, the mice were only perfused with PBS, afterwards mouse brains were dissected and frozen in liquid nitrogen or on dry ice.

4.8 Hematoxylin and Eosin staining

Paraffin tissue sections were deparaffinized in xylene for 30 min and rehydrated through a series of EtOH/water solutions from 100 %, 96 %, 80 %, and 70 %, followed by dH2O, submerging the sections for 2 min each step. The rehydrated sections were stained with Harris' hematoxylin (J.T. Baker, Biosystems Switzerland AG, Muttenz, CH), for 0.5 min, washed twice in dH2O and differentiated in 0.2 % HCl diluted in 70 % ethanol. Afterwards the Sections were stained in 1 % Erythrosine B (RAL DIAGNOSTICS, Biosystems Switzerland AG, Muttenz, CH), a stain closely resembling eosin Y, washed in dH2O and dehydrated through an ascending series of EtOH water solutions, form 70 %, 80 %, 100 %, followed by xylene. Subsequently the sections were mounted using Pertex (Histolab Products AB, Biosystems Switzerland AG, Muttenz, CH).

4.9 Gallyas silver staining

Tissue sections were silver-impregnated following the method of Gallyas-Braak to visualize filamentous tau pathology (Gallyas, 1971; Braak *et al.*, 1988). Paraffin tissue sections were deparaffinized and rehydrated as described above. Afterwards the sections were incubated in 3 % periodic acid (w/v) for 30 min, rinsed in dH2O and incubated in alkaline silver solution (1 M NaOH, 0.6 M KI, 2 mM AgNO3) for 10 min.

Subsequently the sections were developed by mixing developer solution 1 (0.5 M Na2CO3), solution 2 (24 mM NH4NO3, 12 mM AgNO3, 3.5 mM H4[Si(W3O10)4]), and solution 3 (24 mM NH4NO3, 12 mM AgNO3, 3.5 mM H4[Si(W3O10)4], 3 % formaldehyde) in a ratio of 30:9:21 immediately before use, the sections were developed for ca. 25 min, simultaneous to monitoring of the reaction process.

The developing was stopped via incubation in 0.5 % acetic acid for 30 min and fixed via incubation in 5 % Na2S2O3 for 3 min. The silver stained sections were rinsed in dH2O, counterstained using hematoxylin and Eosin, differentiated, dehydrated, and embedded as described on the previous page.

4.10 Immunohistochemistry and Western blots

For tau immunohistochemistry, the monoclonal antibodies AT8 (1:1000, Thermo Scientific, Waltham, US), and HT7 (1:1500, Thermo Scientific, Waltham, US) were used as described previously (Ozcelik *et al.*, 2016). For Aβ immunohistochemistry M0872 antibody (1:10 Agilent Technologies, Santa Clara, US) was used. Paraffin tissue sections were deparaffinized and rehydrated as described for Hematoxylin and Eosin staining. For epitope retrieval the slides were placed in ProTaqs citrate buffer with pH 6 (BIOCYC, Potsdam, DE) and microwaved for 30 min. at 90 °C. The secondary antibodies and peroxidase (HRP) substrate were from Vector Laboratories, Burlingame, CA (Vectastain ABC kit) and used according to manufacturer's protocol.

For seedings with Sup35NM, -5TyrSup35NM and brain homogenate from B6 mice, for quantification of the CA3 region (Lorente de Nó, 1934), a total of 3 brain tissue sections were analyzed (at levels -2.6 mm, -2.4 mm and -2.2 mm from Bregma) per mouse and staining. Bregma levels were determined by visual comparison with the Mouse Brain Atlas (Franklin and Paxinos, 2007). The number of AT8 positive neurons and Gallyas-Braak silver stained neurons was counted by two raters (M.F., A.M.) on 10X magnified, randomly labelled images, taken with a BX43 Upright Microscope (Olympus, Shinjuku, Japan). The mean of the neuron numbers obtained by the two raters was used for statistical analysis.

For a few stainings, only 2 sections next to the indicated Bregma levels were well preserved and usable for quantification (4 sections lacking for AT8 stainings, 3 sections lacking for the Gallyas-Braak stainings). Due to fixation artifacts, which interfered with immunohistochemistry but not with Gallyas-Braak silver staining, AT8 immunohistochemistry was not quantifiable for one mouse of the -5TyrSup35NM group, and two mice of the Sup35NM group.

For Western blots brain tissue was homogenized as described in 3.3. Western blots were then performed under non-reducing conditions by using samples composed of an appropriate amount of protein, 4X NuPAGE LDS sample buffer (Thermo Scientific, Waltham, US) and deionized water. Additional application of 10X NuPAGE reducing agent (Thermo Scientific, Waltham, US) was used to obtain reducing conditions.

4.11 Dot blots

For dot blots, serum of 3-months-old P301Shet and P301SxTAU62on-off mice was separated from the clot by centrifuging the samples at 1000 rpm for 15 minutes at 4 °C, with the remaining supernatant aliquoted and stored at -20 °C, for later usage. Then, a nitrocellulose membrane (Bio-Rad Laboratories, Inc., Hercules, USA) was divided in a grid to allow later incubations with 3-months-old P301Shet and P301SxTAU62on-off sera and HT7 antibody as positive control at 1:1000 dilutions. 0.2 mg/ml of 2N4R wild-type tau monomers were applied on a 4x3 grid. Next, unspecific binding epitopes were blocked with 5% BSA in PBS-Tween, followed by a 30-minutes-long incubation at room temperature with previously extracted sera from the mice and HT7 antibody as positive control. After washing with PBS-Tween, the membrane was incubated with HRP-conjugated anti-mouse secondary antibody at room temperature, washed again in PBS-Tween, and detected by ECL (GE Healthcare, Little Chalfont, UK).

4.12 Statistical analysis

Statistical analysis for the project "Cross-seeding of Alzheimer-related prion-like proteins by non-mammalian prions"

We first compared the ipsilateral (inoculated) side to the contralateral (non-inoculated) side of Sup35NM injected mice. In order to estimate the number of AT8 or Gallyas-Braak stain positive neurons in their hippocampal CA3 fields, the mean neuron counts per mouse of the ipsilateral side were compared by paired T-tests to the contralateral side.
Next, the estimated mean neuron counts per mouse in the ipsilateral CA3 field of P301S tau transgenic mice inoculated with either B6-bh, -5TyrSup35NM or Sup35NM were compared.
This comparison between study groups was done using a one-way ANOVA.

P-values were adjusted for multiple comparisons using Tukey's test. A p-value < 0.05 was considered as significant. All evaluations were done using the statistical software "R" (www.r-project.org). Mean values and standard deviations are shown in figures.

Statistical analysis for the project "Severe oligomeric tau toxicity can be reversed without long-term sequelae"

To evaluate behavioral test results statistically, one-way analysis of variance (ANOVA) followed by post-hoc Student's t-tests and Bonferroni correction for multiple comparisons were applied. P-values < 0.05 were considered significant. To estimate soluble tau and total tau expression from western blots of 3-months-old and 16-months-old mice, the protein bands were normalized to GAPDH protein standard, and quantified using ImageJ software; generated mean ratio values were compared by Student's t-test. To determine the effect of early neurotoxic stress in aged mice, AT8 and Gallyas-positive neurons were semi-quantitatively assessed in brainstem regions. Three sections per animal were analyzed. The total area analyzed per animal was comparable between animals (AT8: P-value = 0,34, and Gallyas: P-value = 0,91). The average count/area ratios were obtained. P-values calculated by Student's t-tests were interpreted exploratory and not adjusted for multiple comparisons; P-values < 0.05 were considered significant. Box plots for figures 2 to 5 were generated with R software. The lower and upper hinges correspond to the first and third quartiles (25th and 75th percentiles). The upper whisker extends from the hinge to the largest value no further than 1.5 times IQR from the hinge (where IQR is the inter-quartile range, or distance between the first and third quartiles). The lower whisker extends from the hinge to the smallest value at most 1.5 times IQR of the hinge. Data beyond the end of the whiskers are called "outlying" points and are plotted individually.

4.13 In vitro seedings

For *in vitro* seedings, recombinant 2N4R wild-type tau (ab84700, Abcam (Cambridge, UK)) and recombinant 2N4R P301S mutant tau (009-001-U01, Rockland Immunochemicals Inc. (Limerick, US)) were used together with the Sup35NM seeds and -5TyrSup35NM monomers described under 3.4 and 3.5.

The buffer of tau monomers was exchanged to Dulbecco's phosphate-buffered saline (DPBS) without calcium and magnesium (D8537, Merck KGaA (Darmstadt, D)) with the use of Microspin™ G-50 columns (GE27-5330-01, GE Healthcare (Chicago, US)).

Sup35NM seeds were created from filaments by sonication (cycle: 0.2 s, amplitude: 100%, 4x 20 s with 2 minutes breaks in between) in a UP200St tube sonicator (Hielscher Ultrasonics GmbH (Teltow, D)). The seeds were filtered with 0.22 µm filter centrifuge tubes to exclude longer filaments.

Tau monomers at a final concentration of 0.1 mg/ml were mixed with 0.027 mg/ml of seeds in DPBS containing 0.02% sodium azide and 0.02 mg/ml heparin (H3393, Merck KGaA (Darmstadt, D)). Samples were incubated at 37 °C on an overhead rotator at 30 RPM. Seeding experiments were replicated three times.

Seeds from Sup35NM induced 2N4R wild-type tau filaments were created by sonication in a similar manner as described above (cycle: 0.2 s, amplitude: 100%, 4x 5 minutes with 5 minutes breaks in between). Tau seeds were diluted 1:3 in 2N4R wild-type tau monomers for re-seeding experiments.

4.14 Transmission electron microscopy

The *in vitro* samples were negatively stained and studied by transmission electron microscopy (TEM) at different time points. 4 µl of sample were adsorbed for 60 s on glow-discharged copper grids that were coated with 2% parlodion and a continuous carbon film.

Samples were blotted with filter paper, washed in three drops of distilled water and stained with 2 droplets of 2% uranyl acetate solution for 20 s, followed by blotting and air-drying.

Labelling of tau filaments with immuno-gold was adapted from Goedert et al. (Goedert *et al.*, 1992). Samples were adsorbed on TEM grids as described before. Grids were laid face down on a drop of 0.1% gelatin (G7041, Merck KGaA (Darmstadt, D)) in DPBS and incubated for 10 min at room temperature. The grids were blotted with filter paper and transferred to a droplet of HT7 anti-tau antibody (MN1000, Thermo Fisher Scientific (Waltham, US), 1:20 dilution in DPBS) and incubated for 60 min at room temperature.

After blotting and washing in 3 drops of gelatin solution the grid was placed on a droplet of anti-mouse antibody conjugated to 10 nm gold beads (EM. GAF10, BBI Solutions (Crumlin, UK)) and incubated for 60 min at room temperature. The grid was blotted and negatively stained with two droplets of uranyl acetate as described above.

Samples were imaged with FEI Tecnai T12 (operated at 120 kV) and FEI Tecnai G2 Spirit (operated at 80 kV) transmission electron microscopes (Thermo Fisher Scientific (Waltham, US)) equipped with TVIPS TemCam-F416 (Tietz Video and Image Processing Systems GmbH (Gauting, D)) and EMSIS VELETA (EMSIS GmbH (Münster, D)) cameras, respectively.

Measurements on the electron micrographs were done with the imageJ distribution Fiji (https://fiji.sc/). For Sup35NM seeded 2N4R wild-type tau, periodicity of the wave pattern was measured and used to calculate mean and standard deviation.

4.15 Genotyping

Tense buffer

Compound	Concentration	Supplier
Tris	100 mM (pH 8.5)	Biomol
EDTA	5 mM	Fluka BioChemika
NaCl	200 mM	Merck
SDS	0.2%	Bio Rad

For sample digestion the thermo shaker was set at 55 °C and 750 µl of tense buffer and 3,75µl of Proteinase K were added to each sample. The samples were incubated overnight (or at least for 4 hours) at 55 °C and 600 rpm.

For DNA Isolation the samples were centrifuged at 4 °C and 14000 g for 5 min. A new tube for each sample was labelled and filled with 750 µl of isopropanol. Subsequently the supernatant was taken from each sample and added to the respective isopropanol tube. The tubes were then centrifuged at 4 °C and 14000 g for 10 min.

Afterwards the supernatant was discharged and 200 µl of 75% ethanol were added to each pallet. Next the samples were centrifuged at 4 °C and 14000 g for 10 min. Afterwards the supernatant was discharged and 250 µl of sterile water were added to each tube. Subsequently the samples were incubated for 1h at 50 °C without shaking.

For PCR 2.5 µl of each DNA sample were added to a PCR tube containing 22.5 µl of PCR mix (see first table below). The primers and PCR programs for the Δtau allele and 4R tau allele (P301S tau detection) are sown below and on the next page.

For Gel electrophoresis 3 gels were prepared by mixing 300 ml of TAE-buffer with 4.5g of agarose in an Erlenmeyer Flask and heating the mixture in the microwave at maximum temp. for 4 min. Afterwards the mixture was cooled down for a couple minutes and 15 µl of cyber save were added. 3 comps were placed in the gel chamber and the chamber was filled with the liquid gel. After solidifying the resulting gel was cut into 3 gels. For immediate use the gels were put in an electrophoresis chamber filled with TAE-buffer, 5 µl of loading dye were added to each sample and the samples were loaded to the gel. The gel was run in TAE-buffer for 60 min. at 150V (90 min. at 150V if the gel is run in TBE-buffer). For storage the gels were stored in TAE-buffer at 4 °C in the fridge.

PCR mix

12,5 µl Qiagen PCR Master mix	Qiagen
4 µl sterile water	Qiagen
2,5 µl forward primer	Thermo Scientific
2,5 µl reverse primer	Thermo Scientific
1 µl DMSO (add last)	Merck

Primers

Primer names	Forward primer	Reverse Primer
TauF151 (for Δtau detection)	GTG GAT CTC AAG CCC TCA AG	GGC GAC TTG GGT GGA GTA
P301S (for 4R tau detection)	GGT TTT TGC TGG AAT CCT GG	GGA GTT CGA AGT GAT GGA AG

PCR programs

TauF151 program	P301S program
Step 1: 95°C for 2 min	Step 1: 95°C for 4 min
Step 2: 95°C for 1 min	Step 2: 95°C for 1 min
Step 3: 60°C for 1 min	Step 3: 60°C for 1 min
Step 4: 72°C for 2 min	Step 4: 72°C for 3 min
Step 5: 72°C for 10 min (final extension)	Step 5: 72°C for 10 min (final extension)
Steps 2-4 were repeated for 30 cycles	Steps 2-4 were repeated for 30 cycles

5 Discussion

The aging of society was associated with a rapid increase in people suffering from dementia with Alzheimer disease (AD) as its predominant form. The case numbers of AD have increased from a few cases per country at the beginning of the 20th century to a current state where every 7 seconds somebody in the world is diagnosed with AD (Cornutiu, 2015) and these numbers are predicted to continue their dramatic increase in the near future (Gaugler *et al.*, 2016). Between 2000 and 2018, deaths resulting from stroke, and heart disease decreased in the US, while reported deaths from AD increased by 146.2% ('2020 Alzheimer ' s disease facts and figures', 2020). In AD and other neurodegenerative proteinopathies with amyloid aggregation most of the cases appear sporadic. To find the original cause for AD and other neurological diseases would be an extremely important step towards a better understanding of the pathological mechanisms of said diseases and an enormous help for the development of preventive or curative approaches. Tau is a hallmark protein of AD and other neurological diseases called tauopathies. Several studies have shown, that tau has prion-like properties, it was therefore termed a prionoid (Scheckel and Aguzzi, 2018). In tauopathies caused by a specific genetic background, disease associated mutations increase the ability of tau to aggregate and build amyloids. The resulting amyloid fibers consist of ordered protein assemblies with abundant cross beta sheet structures (Barghorn *et al.*, 2000; Knowles, Vendruscolo and Dobson, 2014). This supports the idea of tau aggregation as a crucial step in pathogenesis. Moreover tau deposition predicts brain atrophy in AD patients with the strongest correlation being found in young patients (La Joie *et al.*, 2020). Additionally, in seeding trails with transgenic mice, tau stains with different conformations caused different levels of pathology (Kaufman *et al.*, 2016). This shows the importance of tau structure in disease induction and tau spreading. And indeed, tau fibrils with different conformations where also found in patients with different tauopathies (Fitzpatrick *et al.*, 2017; Falcon *et al.*, 2018; Zhang *et al.*, 2019). The initial reason for the aggregation and amyloid formation of tau in sporadic and genetic cases of AD and other neurological diseases is still unknown. Sup35 is a prion protein in yeast which also changes its confirmation and forms in-register beta sheet structured amyloids, with Sup35NM constituting its prion domain (Gorkovskiy *et al.*, 2014). Similar to tau, Sup35NM forms fibrils with different confirmations and these different fibril confirmations lead to a different level of infectivity of the respective Sup35NM strains (Ohhashi *et al.*, 2010). We were now able to show that Sup35NM promotes tau aggregation *in vitro* as well as *in vivo*. We were therefore able to demonstrate that in principle a natural prion from a species present in the human microbiome is able to cause the aggregation of a prionoid like tau protein, thereby starting a pathological cascade of conformational changes like the one reported in tauopathies including AD.

Strikingly, in a first trial the inoculation of App23 mice, overexpressing human amyloid precursor protein (APP) bearing the pathogenic Swedish mutation (Sturchler-Pierrat *et al.*, 1997), with Sup35NM solution, also led to an acceleration and accentuation of the formation of Aβ aggregates in the brains of those mice.

Furthermore, in a mouse model for amyloid protein A amyloidosis seeding with Sup35 fibrils resulted in an accelerated amyloid formation of amyloid protein A (AA) (Lundmark, Westermark and Olse, 2005). This is additional prove that the seeding potential of prions is mainly based on structural similarities, in this case in-register beta sheet amyloids, and not similarities in the DNA sequence of the respective proteins.

Our results are also in coherence with the fact, that in AD early tau aggregation occurs in the olfactory bulb and approximately 90% of AD patients show olfactory dysfunction as an early symptom, which correlates with a higher tau burden in the brains of prodromal AD patients (Doty, 2003; Arnold *et al.*, 2010; Risacher *et al.*, 2017). The nasal sensors are in close contact to exogenous agents and the microbiome in the nasopharyngeal cavity and they are anatomically connected with the limbic system, which is also affected early in AD (Ulrich, 1985). Therefore, the olfactory system delivers a potential gateway for exogenous prions and other xenobiotics to enter the central nervous system without the need to pass the blood brain barrier (Brai and Alberi, 2018; Rey, Wesson and Brundin, 2018). Olfactory deficits are also present in other neurodegenerative diseases especially Parkinson and Huntington disease (Moberg and Doty, 1997; Takeda *et al.*, 2014). In Parkinson disease impaired olfaction is also one of the earliest signs often predating the diagnosis by 2 years (Ross *et al.*, 2006). Additionally, researchers could show that injection of α-synuclein fibers into the olfactory bulbs of WT mice results in olfactory deficits and a spreading of α-synuclein which is reminiscent of the pattern observed in early stages of Parkinson (Rey *et al.*, 2016)

The fact that, pathological protein aggregates affect olfactory regions prior to other regions in the brain, suggests that the olfactory system might be particularly vulnerable for protein aggregation in neurodegenerative diseases (Rey, Wesson and Brundin, 2018). Due to this evidence tests for olfactory function have been included in the diagnostic tools for Parkinson and AD (Devanand *et al.*, 2000, 2010; Miller and O'Callaghan, 2015; Yoon *et al.*, 2015; Lafaille-Magnan *et al.*, 2017). Further evidence for the involvement of fungal material in AD comes from a study where researchers were able to show fungal infection in 100% of the central nervous system (CNS) samples form AD patients they examined while at the same time 0% of the examined CNS samples of healthy control patients showed any signs of fungal infection (Pisa *et al.*, 2015). Thereby, fungal material could be detected extracellularly as well as in inside the neurons of AD patients.

The fungal species detected in the brains of the patients include *Saccharomyces cerevisiae*, *Candida albicans* and *Malassezia globosa*. Fungal material was also found in the blood of AD patients (Alonso *et al.*, 2014; Pisa *et al.*, 2015) were it could interact with Aβ and influence vascular disease as indicated by our *in vivo* trials with Aβ and Sup35NM. Depending on the study up to 90% of AD patients exhibit cerebrovascular pathology (Kalaria, Akinyemi and Ihara, 2012; Brickman *et al.*, 2018), therefore a further investigation of the interaction of fungal material with Aβ or other compounds present in the blood of AD patients could also be of particular interest.

An amyloid inducing interaction between two proteins of human origin was shown for α-synuclein and islet amyloid polypeptide (IAPP) aka amylin (Valbuena, Villegas and Azcarate, 2018). Under physiological conditions IAPP plays an adaptive role in metabolism and glucose homeostasis, by helping to control gastric emptying, suppressing the release of glucagon and insulin and helping to regulate satiety (Westermark, Andersson and Westermark, 2011). Phosphorylated α-synuclein deposits were found in the pancreatic β cells of 93% of Parkinson patients, 68% of diabetes mellitus patients and 17% of healthy controls. Interestingly while α-synuclein and IAPP where co-expressed in the pancreatic β cells of all participants an interaction between the two proteins occurred only in subjects showing cytoplasmic deposits of phosphorylated α-synuclein in healthy as well as in diseased participants. In *in vitro* seeding trials preformed IAPP amyloid seeds accelerated the formation of α-synuclein fibrils while preformed α-synuclein amyloid seeds actually inhibited the formation of IAPP fibrils (Horvath and Wittung-Stafshede, 2016). This is in line with the fact that patients with type 2 diabetes (T2D) have a higher risk to develop Parkinson while Parkinson patients where not shown to have a higher risk to develop T2D (Xu *et al.*, 2011; Santiago and Potashkin, 2014; Pagano *et al.*, 2018) This indicates, that fibrils from a protein with a faster aggregation rate are able to accelerate the aggregation of monomers from a protein that has a slower aggregation rate. This fits well with our results about Sup35NM and tau aggregation *in vitro* where after 3 days of incubation there was a brought mixture of Sup35NM fibrils visible for Sup35NM while unseeded WT-tau did not show any fibrils at this time point, unless it was seeded with Sup35NM fibrils.

Another potential way on how prions from the microbiome could influence the aggregation of prionoids with key roles in neurodegeneration is the migration of prions from the gut microbiome to the brain via the vagus nerve. And indeed in a model for Parkinson the injection of α-synuclein fibrils into the gut of mice led to the misfolding of endogenous α-synuclein to its pathological form in the brain, while dissection of the vagus nerve prevented any cerebral α-synuclein misfolding (Kim *et al.*, 2019).

The results from Parkinson patients are less clear, while a Swedish study found a small protective effect for vagus nerve dissection starting 5 years post-surgery (Liu *et al.*, 2017) a Danish study on the same topic was not able to find any significant difference between patients and controls with an intact vagus nerve (Tysnes *et al.*, 2015). This suggests, that the vagus nerve is not the only route which can be used by α-synuclein in order to enter the brain.

More evidence for gut derived α-synuclein seeding comes from a recent study in which scientists could show that gastrointestinal exposure of mice overexpressing human α-synuclein to bacterial amyloid protein curli leads to increased α-synuclein aggregation in the gut and the brain of those mice (Sampson *et al.*, 2020). This is further evidence for the ability of non-human prions to seed the aggregation of human prionoids involved in the development of neurodegenerative diseases. Interestingly, in the same study it was shown, that curli is unable to seed tau aggregation.

Together with the results from the one directional seeding of IAPP and α-synulein this opens the perspective for a hierarchic prion cross-seeding system, separating highly cross-seeding competent prions from others, which might be more “seedable”.

Sup35 is a translation termination factor and acts as a suppressor of nonsense mutations in *Saccharomyces cerevisiae* (King *et al.*, 1997; Kushnirov, 2000). Sup35, in its prion form, occurs in wild yeasts on grapes and on *Saccharomyces cerevisiae* strains of commercial wine yeast (Halfmann *et al.*, 2012). *Saccharomyces cerevisiae* strains able to form Sup35 amyloid aggregates were also found in yeast samples from some breweries and some commercial fruit (Kelly, Busby and Wickner, 2014).

Interestingly, the exposure of millions of humans to the bovine spongiform encephalopathy (BSE) only resulted in a small number of patients with variant Creuzfeld-Jakob disease (vCJD). It is estimated that in the UK alone 10 million individuals consumed prion contaminated beef (Chen and Wang, 2014), however only 177 cases of vCJD were reported in the UK, with the majority of cases detected between 1995 and 2005 and only a few cases from 2012 to 2016 (Comoy *et al.*, 2017). In contrast to this low case numbers, in an anonymized survey in the UK disease-associated prion protein (PrP^{d}) was found in 16 out of 32000 samples from human appendices (Noel Gill *et al.*, 2013). This indicates an overall prevalence of 493 per million for the UK population and therefore a significant number of PrP^{d} carriers without any symptoms of vCJD. In accordance with these results in another study scientist discovered small amounts of the pathogenic conformer of PrP in the brain autopsies of healthy volunteers (Yuan *et al.*, 2006). Additionally, acquired human prion diseases often have very long incubation periods reaching up to three decades in patients with iatrogenic PrP^{d} infections or Aβ transmissions by contaminated growth hormone or gonadotropin and even five decades in people infected with Kuru via cannibalism (Collinge *et al.*, 2006; Jaunmuktane *et al.*, 2015; Purro *et al.*, 2018).

Altogether these results suggest that a certain event is needed to trigger the spread of pathological prion proteins in the brains of carriers.

In this context, exposure to microbiotic or exogenous non-mammalian prions early in life would be followed by a long incubation period in sporadic AD. Thus, our putative theory might explain the relationship between the biology of aging and the biology of AD pathology.

In theory, it could be that in many cases prions like Sup35 simply don't get into direct contact with proteins like tau in the brain of their carriers for a very long time period, which would be necessary for them to induce a conformational change. Additionally, it could also be that the interaction of prions with their target proteins and the spreading of the resulting misfolded prionoids could be influenced by certain immunological events. In analogy, for herpes simplex virus type 1 (HSV-1) the site of entry is a critical factor for the development of a latent or active infection (Hafezi et al., 2012) and the deprivation of nerve growth factor leads to the reactivation of herpes virus from the latent phase (Wilcox and Johnson, 1987). There seems to be a connection between inflammation and AD however, with all the research done on this specific sub topic so far it is still not known if inflammation is a cause, contributor, or secondary phenomenon in AD and trials with anti-inflammatory drugs have been a major disappointment so far, as they have been for virtually all other therapeutics that have attempted to address the underlying pathogenesis of AD (Wyss-Coray and Rogers, 2012; Ozben and Ozben, 2019). Interestingly, some people suffering from a long-lasting active inflammatory disease like rheumatoid arthritis, tuberculosis and malaria develop amyloid protein A (AA) amyloidosis a disease where a N-terminal fragment of serum AA forms β-sheet fibrils that are deposited predominantly in kidney liver and spleen (McAdam, 1978; Johan *et al.*, 1998; Solomon *et al.*, 2007). Why some of these patients develop AA amyloidosis and others not is unknown so far. However, it was shown that amyloid fibrils are able to highly accelerate the formation of AA fibrils in a mouse model. These fibrils include Sup35 and AA amyloid deposits from duck- or goose derived foie gras (Lundmark, Westermark and Olse, 2005; Solomon *et al.*, 2007). Therefore, these results give a potential hint for an interaction between inflammation and prion-like seeding where one event fuels the other.

In light of this our data primarily call for the advanced analysis of the cross-species cross-seeding capacity of Sup35 and other non-human prions, the search for such fungal, bacterial or plant prions in samples of the human microbiome, as well as the further study of cross-seeding effects of Sup35 on amyloid-β and other human prionoids, e.g., α-synuclein. In this context it would also make sense to analyze the tau filaments, resulting from Sup35NM seeding, with cryo-EM.

Therefore, the structure of the filaments could be determined and compared to the already known structures of tau fibrils from different tauopathies (Fitzpatrick *et al.*, 2017; Falcon *et al.*, 2018; Zhang *et al.*, 2019), consequently, allowing to find out if the Sup35NM induced curly tau structure is a new structure and if it has common features with the pathological tau detected in tauopathies. Understanding the initiation of prion formation in sporadic neurodegenerative disorders will be pivotal for the long-awaited generation of preventive or curative approaches for these devastating disorders.

When thinking about a potential treatment for AD another important point to consider is the potential long term effect of the neurotoxic stress caused by misfolded pathological proteins like tau. In order to address this question, we studied the long-term fitness of P301SxTau62 (P62) mice after their recovery from a reversible tau induced paresis. The paresis had been caused by the co-expression of full-length P301S mutant tau together with a wild-type 3R tau fragment from AS 151-421 (Δtau). After the expression of Δtau had been stopped at the age of 3 weeks the mice recovered completely from their severe hind limp paresis in the timespan of another 3 weeks besides the continued expression of P301S tau. Interestingly, in old age the recovered P62 mice did show a slightly better motoric fitness as their heterozygous littermates, only expressing P301S tau since their birth, and lower levels of tau pathology in their brains. This might be due to the fact that paralyzed P62 mice only have oligomeric tau forms in their brain while fibrillary tau is absent. When we inoculated the brains of Alz17 mice, expressing the longest human tau isoform and not developing filamentous tau aggregates by themselves, with brain stem homogenates of paralyzed P62 mice no formation of tau fibrils was observed.

In contrast, control seedings with the brain stem homogenates of aged P301S mice, containing fibrillary tau, induced a granular focal tau pathology, primarily in the ipsilateral dorsal fornix above CA1 and the fimbria. This is further evidence, that the structure of tau aggregates plays a crucial role when it comes to tau pathology spreading in the brain via prion-like template transmission.

It also speaks for the development of therapies which target tau before the formation of tau fibrils was initiated and asks for caution regarding the use of seeding based assays for the detection of very early preclinical disease manifestations of tauopathies.

6 Conclusions

Here we demonstrate that the yeast Sup35NM prion domain can promote the formation of tau aggregation *in vitro* and *in vivo.* This provides first evidence for heterotypic cross-species seeding of a human neurodegenerative prion-like protein by a yeast prion. This calls for further analysis of the cross-species seeding competence of non-human (fungal, bacterial, viral) prions and prion-like neurodegenerative proteins, as well as research on the presence of non-human prions in the human microbiome.

In the second project we demonstrate that high molecular weight tau oligomers can provoke a severe, but reversible neurotoxicity. Rescued mice do not suffer from long-term sequelae, what augurs well for early therapeutic interventions targeting oligomeric tau forms. Furthermore, we confirm that these early toxic tau species lack classical seeding competence. Confirming the importance of an appropriate β-amyloid structure for tau seeding and spreading. This asks for caution regarding the use of seeding based assays for the detection of very early preclinical disease manifestations of tauopathies. It also warrants for a deepened analysis of early, non-fibrillary toxic tau forms in the future

Altogether there are still many open questions concerning AD and neurodegenerative diseases in general. However, there is immense evidence, that the conformation of tau plays a crucial role in the development of AD and other tauopathies (Arnold *et al.*, 2013; Clavaguera *et al.*, 2013; Sanders *et al.*, 2014; Kaufman *et al.*, 2016; Falcon *et al.*, 2018; Scheckel and Aguzzi, 2018; Zhang *et al.*, 2019).

As tau plays several important roles in the development and maintenance of brain function (Lee and Rook, 1992; Hernandez and Avila, 2007) a simple deactivation or clearance of tau cannot be used to cure these diseases. Therefore, the best strategy for the prevention of AD seems to be the conservation of the functional physiological structure of tau. If this can be achieved by the removal of exogenous prions, which deliver a template for pathological tau seeding, the modification of post-translational modifications of tau or a combination of both has to be further investigated.

It will still take at least some years to answer the questions concerning the origin, the progression and the potential healing of AD. In this context I am proud and happy that we were able to contribute two small pieces to this very challenging puzzle.

7 Abbreviations

×R	× microtubule binding repeats
3R	3 microtubule binding repeats
AA	amyloid protein A
AcMNPV	Autographa californica multiple nucleopolyhedrovirus
AD	Alzheimer disease
AEP	asparagine endopeptidase
APOE-ε4	apolipoprotein E, type 4 allele
APP	amyloid precursor protein
APP	amyloid precursor protein
Aβ	amyloid-beta
B6 mice	C57BL/6 mice
BBB	blood brain barrier
BSE	bovine spongiform encephalopathy
CAA	cerebral amyloid angiopathy
CBD	cortico basal degeneration
CBP	cAMP-response element binding protein (CREB)-binding protein
Cdk5	cyclin-dependent kinase-5
CJD	Creutzfeldt-Jakob disease
CK1	casein kinase 1
CNS	central nervous system
CTE	Chronic traumatic encephalopathy
DPBS	Dulbecco's phosphate-buffered saline
FAD	familial Alzheimer’s disease
FTDP-17	frontotemporal dementia with parkinsonism linked to chromosome 17
FTLD-tau	frontotemporal lobar degeneration-tau
GSK-3	glycogen synthase kinase-3
HDAC6	histone deacetylase 6
HSV-1	herpes simplex virus type 1
htau	human tau
IAPP	islet amyloid polypeptide

IPTG	Isopropyl β-d-1-thiogalactopyranoside
LDPrD	prion-like domain of Arabidopsis protein Luminidependens
MAPKs	mitogen-activated protein kinases
MAPs	microtubule-associated proteins
MAPT	Microtubule-associated protein tau
MARKs	microtubule affinity-regulating kinases
MT	microtubule
N279	asparagine residue 279
NFTs	neurofibrillary tangles
NTs	neuropil threads
O-GlcNAc	N-acetylglucosamine
O-GlcNAcylation	addition of β-linked N-acetylglucosamine
P62	P301SxTau62
PD	Prion disease
PDPKs	proline-directed protein kinases
PEP	postencephalitic parkinsonism
PiD	Pick's, disease
PKA	cAMP-dependent protein kinase A
PMSF	phenylmethylsulfonyl fluoride
PP2A	protein phosphatase 2A
PP5	phosphatase 5
PrP	prion protein
PrP^{d}	disease-associated prion protein
PrPSc	PrPs β-sheet structured amyloid
PS1	presenilin 1
PS2	presenilin 2
PSA	puromycin-sensitive aminopeptidase
PSP	progressive supranuclear palsy
SAD	sporadic Alzheimer’s disease
SIRT1	sirtuin 1
T2D	type 2 diabetes
TEM	transmission electron microscopy
vCJD	variant Creuzfeld-Jakob disease

WT	wild type
Δtau	human tau fragment from amino acid 151-421

8 Acknowledgments

First, I would like to thank David Winkler for giving me the opportunity to join his group and for supervising my research efforts. I would also like to thank you for your support, advice and encouragement.

Special thanks goes out to my group member Alfonso Martinisi. You were always a great team mate. Thanks for your help and the talks about science and many other aspects of life.

I would like to express my great appreciation to Henning Stahlberg and Cedric Leu for the enjoyable collaboration and great communication during our projects.

I would like to express my gratitude to Frederik Sprenger, Lisa Restelli and Gabriel Schweighauser for their help at the beginning of my PhD.

I am grateful to our collaborators, Reed Wickner and Michel Goedert, for their expertise and for providing the Sup35NM plasmid respectively the P301S tau transgenic mice.

I thank Christoph Handschin and Paolo Paganetti for supporting my dissertation as members of the Doctoral Committee.

I would like to offer my special thanks to the staff at the ZLF animal facility for their support with the mice.

I also want to thank the all my colleagues at the Institute of Pathology. In my daily work, I have been blessed with friendly and supportive people providing a great atmosphere in the lab. I know that is not something you can take for granted and I really appreciate it.

Finally, I want to thank my great and lovely family and dear friends for their presence and support in my life.

9 References

'2020 Alzheimer ' s disease facts and figures' (2020) *Alzheimer's and Dementia*, pp. 391–460. doi: 10.1002/alz.12068.

Adams, S. J. *et al.* (2009) 'Overexpression of wild-type murine tau results in progressive tauopathy and neurodegeneration', *American Journal of Pathology*, 175(4), pp. 1598–1609. doi: 10.2353/ajpath.2009.090462.

Ahmed, Z. *et al.* (2014) 'A novel in vivo model of tau propagation with rapid and progressive neurofibrillary tangle pathology: The pattern of spread is determined by connectivity, not proximity', *Acta Neuropathologica*, 127(5), pp. 667–683. doi: 10.1007/s00401-014-1254-6.

Aizenstein, H. J. *et al.* (2008) 'Impairment Among the Elderly', *Archives of Neurology*, 65(11), pp. 1509–1517. doi: 10.1001/archneur.65.11.1509.Frequent.

Allen, B. *et al.* (2002) 'Abundant tau filaments and nonapoptotic neurodegeneration in transgenic mice expressing human P301S tau protein', *Journal of Neuroscience*. Soc Neuroscience, 22(21), pp. 9340–9351.

Alonso, R. *et al.* (2014) 'Alzheimer's disease and disseminated mycoses', *European Journal of Clinical Microbiology and Infectious Diseases*, 33(7), pp. 1125–1132. doi: 10.1007/s10096-013-2045-z.

Andreadis, A., Broderick, J. A. and Kosik, K. S. (1995) 'Relative exon affinities and suboptimal splice site signals lead to non-equivalence of two cassette exons', *Nucleic acids research*. Oxford University Press, 23(17), pp. 3585–3593.

Arakhamia, T. *et al.* (2020) 'Posttranslational Modifications Mediate the Structural Diversity of Tauopathy Strains Article Posttranslational Modifications Mediate the Structural Diversity of Tauopathy Strains', *Cell*. Elsevier Inc., 180(4), pp. 633-644.e12. doi: 10.1016/j.cell.2020.01.027.

Arendt, T., Stieler, Jens T and Holzer, M. (2016) 'Tau and tauopathies', *Brain Research Bulletin*, 126, pp. 238–292. doi: 10.1016/j.brainresbull.2016.08.018.

Arendt, T., Stieler, Jens T. and Holzer, M. (2016) 'Tau and tauopathies', *Brain Research Bulletin*, 126, pp. 238–292. doi: 10.1016/j.brainresbull.2016.08.018.

Arnold, S. E. *et al.* (2010) 'Olfactory epithelium amyloid-β and paired helical filament-tau pathology in Alzheimer disease', *Annals of Neurology*, 67(4), pp. 462–469. doi: 10.1002/ana.21910.

Arnold, S. E. *et al.* (2013) 'Comparative survey of the topographical distribution of signature molecular lesions in major neurodegenerative diseases', *Journal of Comparative Neurology*, 521(18), pp. 4339–4355. doi: 10.1002/cne.23430.

Arrasate, M., Pérez, M. and Avila, J. (2000) 'Tau dephosphorylation at Tau-1 site correlates with its association to cell membrane', *Neurochemical Research*, 25(1), pp. 43–50. doi: 10.1023/A:1007583214722.

Askanas, V. and Engel, W. K. (2008) 'Inclusion-body myositis: Muscle-fiber molecular pathology and possible pathogenic significance of its similarity to Alzheimer's and Parkinson's disease brains', *Acta Neuropathologica*, 116(6), pp. 583–595. doi: 10.1007/s00401-008-0449-0.

Avila, J. (2008) 'Tau kinases and phosphatases.', *Journal of cellular and molecular medicine*, 12(1), pp. 258–9. doi: 10.1111/j.1582-4934.2007.00214.x.

Bäckhed, F. *et al.* (2005) 'Host-bacterial mutualism in the human intestine', *Science*, 307(5717), pp. 1915–1920. doi: 10.1126/science.1104816.

Bäckhed, F. *et al.* (2012) 'Defining a healthy human gut microbiome: Current concepts, future directions, and clinical applications', *Cell Host and Microbe*, 12(5), pp. 611–622. doi: 10.1016/j.chom.2012.10.012.

Barghorn, S. *et al.* (2000) 'Structure, microtubule interactions, and paired helical filament aggregation by tau mutants of frontotemporal dementias', *Biochemistry*, 39(38), pp. 11714–11721. doi: 10.1021/bi000850r.

Basurto-Islas, G. *et al.* (2008) 'Accumulation of aspartic acid421- and glutamic acid 391-cleaved tau in neurofibrillary tangles correlates with progression in Alzheimer disease', *Journal of Neuropathology and Experimental Neurology*, 67(5), pp. 470–483. doi: 10.1097/NEN.0b013e31817275c7.

Baxa, U. *et al.* (2002) 'Mechanism of inactivation on prion conversion of the Saccharomyces cerevisiae Ure2 protein'.

von Bergen, M. *et al.* (2000) 'Assembly of tau protein into Alzheimer paired helical filaments depends on a local sequence motif ((306)VQIVYK(311)) forming beta structure.', *Proceedings of the National Academy of Sciences of the United States of America*, 97(10), pp. 5129–34. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10805776%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC25793.

Bloom, G. S. (2014) 'Amyloid-β and tau: The trigger and bullet in Alzheimer disease pathogenesis', *JAMA Neurology*, 71(4), pp. 505–508. doi: 10.1001/jamaneurol.2013.5847.

Bogaert, D. *et al.* (2011) 'Variability and diversity of nasopharyngeal microbiota in children: A metagenomic analysis', *PLoS ONE*, 6(2). doi: 10.1371/journal.pone.0017035.

Braak, H. *et al.* (1988) 'Silver impregnation of Alzheimer's neurofibrillary changes counterstained for basophilic material and lipofuscin pigment', *Stain technology*. Taylor & Francis, 63(4), pp. 197–200.

Braak, H. *et al.* (2003) 'Idiopathic Parkinson's disease: possible routes by which vulnerable neuronal types may be subject to neuroinvasion by an unknown pathogen', *Journal of neural transmission*. Springer, 110(5), pp. 517–536.

Braak, H. and Braak, E. (1991) 'Neuropathological stageing of Alzheimer-related changes.', *Acta neuropathologica*, 82(4), pp. 239–59. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1759558.

Brai, E. and Alberi, L. (2018) 'Olfaction, among the first senses to develop and decline', *Sensory Nervous System*. BoD–Books on Demand, p. 65.

Brandt, R., Léger, J. and Lee, G. (1995) 'Interaction of tau with the neural plasma membrane mediated by tau's amino-terminal projection domain.', *The Journal of cell biology*. Rockefeller University Press, 131(5), pp. 1327–1340.

Brickman, A. M. *et al.* (2018) 'An MRI measure of degenerative and cerebrovascular pathology in Alzheimer disease', *Neurology*. AAN Enterprises, 91(15), pp. e1402–e1412.

Buée, L. *et al.* (2000) 'Tau protein isoforms, phosphorylation and role in neurodegenerative disorders', *Brain Research Reviews*. Elsevier, 33(1), pp. 95–130.

Cambiazo, V., González, M. and Maccioni, R. B. (1995) 'DMAP-85: A τ-Like Protein from Drosophila melanogaster Larvae', *Journal of neurochemistry*. Wiley Online Library, 64(3), pp. 1288–1297.

Van Cauwenberghe, C., Van Broeckhoven, C. and Sleegers, K. (2016) 'The genetic landscape of Alzheimer disease: clinical implications and perspectives', *Genetics in Medicine*. Nature Publishing Group, 18(5), p. 421.

Chai, C. K. (2007) 'The genetics of Alzheimer's disease', *American Journal of Alzheimer's Disease & Other Dementias®*. Sage Publications Sage CA: Thousand Oaks, CA, 22(1), pp. 37–41.

Chakrabortee, S. *et al.* (2016) 'Luminidependens (LD) is an Arabidopsis protein with prion behavior', *Proceedings of the National Academy of Sciences*, 113(21), pp. 6065–6070. doi: 10.1073/pnas.1604478113.

Chapman, M. R. *et al.* (2002) 'Role of Escherichia coli Curli Operons in Directing Amyloid Fiber Formation', *Science*, 295(February), pp. 851–856.

Chen, C. C. and Wang, Y. H. (2014) 'Estimation of the exposure of the uk population to the bovine spongiform encephalopathy agent through dietary intake during the period 1980 to 1996', *PLoS ONE*, 9(4). doi: 10.1371/journal.pone.0094020.

Chen, J. *et al.* (1992) 'Projection domains of MAP2 and tau determine spacings between microtubules in dendrites and axons', *Nature*, 360(6405), pp. 674–677. doi: 10.1038/360674a0.

Chohan, M. O. *et al.* (2005) 'Hyperphosphorylation-induced self assembly of murine tau: A comparison with human tau', *Journal of Neural Transmission*, 112(8), pp. 1035–1047. doi: 10.1007/s00702-004-0241-9.

Cisternino, S. *et al.* (2003) 'Nonlinear accumulation in the brain of the new taxoid TXD258 following saturation of P-glycoprotein at the blood-brain barrier in mice and rats', *British Journal of Pharmacology*, 138(7), pp. 1367–1375. doi: 10.1038/sj.bjp.0705150.

Clavaguera, F. *et al.* (2009) 'Transmission and spreading of tauopathy in transgenic mouse brain', *Nature Cell Biology*. Nature Publishing Group, 11(7), pp. 909–913. doi: 10.1038/ncb1901.

Clavaguera, F. *et al.* (2013) 'Brain homogenates from human tauopathies induce tau inclusions in mouse brain', *Proceedings of the National Academy of Sciences*, 110(23), pp. 9535–9540. doi: 10.1073/pnas.1301175110.

Cohen, T. J. *et al.* (2013) 'The microtubule-associated tau protein has intrinsic acetyltransferase activity', 20(6), pp. 756–763. doi: 10.1038/nsmb.2555.

Cohen, T. J. *et al.* (2016) 'Intrinsic tau acetylation is coupled to auto-proteolytic tau fragmentation', *PLoS ONE*, 11(7), pp. 1–19. doi: 10.1371/journal.pone.0158470.

Collinge, J. *et al.* (2006) 'Kuru in the 21st century-an acquired human prion disease with very long incubation periods', *Lancet*, 367(9528), pp. 2068–2074. doi: 10.1016/S0140-6736(06)68930-7.

Comoy, E. E. *et al.* (2017) 'Experimental transfusion of variant CJD-infected blood reveals previously uncharacterised prion disorder in mice and macaque', *Nature Communications*. Springer US, 8(1). doi: 10.1038/s41467-017-01347-0.

Cook, C. *et al.* (2014) 'Acetylation of the KXGS motifs in tau is a critical determinant in modulation of tau aggregation and clearance', 23(1), pp. 104–116. doi: 10.1093/hmg/ddt402.

Corbett, G. T. *et al.* (2019) 'PrP is a central player in toxicity mediated by soluble aggregates of neurodegeneration-causing proteins', *Acta Neuropathologica*. Springer Berlin Heidelberg, 139(3), pp. 503–526. doi: 10.1007/s00401-019-02114-9.

Cornutiu, G. (2015) 'The Epidemiological Scale of Alzheimer's Disease', *Journal of Clinical Medicine Research*, 7(9), pp. 657–666. doi: 10.14740/jocmr2106w.

Correas, I., Padilla, R. and Avila, J. (1990) 'The tubulin-binding sequence of brain microtubule-associated proteins, tau and MAP-2, is also involved in actin binding', *Biochemical Journal*. Portland Press Limited, 269(1), pp. 61–64.

Dan, A. *et al.* (2014) 'Extensive deamidation at asparagine residue 279 accounts for weak immunoreactivity of tau with RD4 antibody in Alzheimer's disease brain', *Acta Neuropathologica Communications*, 2(1), pp. 1–9. doi: 10.1186/2051-5960-1-54.

Dando, S. J. *et al.* (2014) 'Pathogens penetrating the central nervous system: Infection pathways and the cellular and molecular mechanisms of invasion', *Clinical Microbiology Reviews*, 27(4), pp. 691–726. doi: 10.1128/CMR.00118-13.

Dehmelt, L. and Halpain, S. (2005) 'The MAP2/Tau family of microtubule-associated proteins', *Genome Biology*, 6(1), pp. 1–10. doi: 10.1186/gb-2004-6-1-204.

Delaere, P. *et al.* (1990) 'Large amounts of neocortical βA4 deposits without neuritic plaques nor tangles in a psychometrically assessed, non-demented person', *Neuroscience letters*. Elsevier, 116(1–2), pp. 87–93.

Derkatch, I. L. *et al.* (2004) 'Effects of Q N-rich , polyQ , and non-polyQ amyloids on the de novo formation of the [PSI ⅋] prion in yeast and aggregation of Sup35 in vitro'.

Devanand, D. P. *et al.* (2000) 'Olfactory deficits in patients with mild cognitive impairment predict Alzheimer's disease at follow-up', *American Journal of Psychiatry*, 157(9), pp. 1399–1405. doi: 10.1176/appi.ajp.157.9.1399.

Devanand, D. P. *et al.* (2010) 'Olfactory identification deficits and MCI in a multi-ethnic elderly community sample', *Neurobiology of Aging*, 31(9), pp. 1593–1600. doi: 10.1016/j.neurobiolaging.2008.09.008.

Dickson, D. W. *et al.* (1992) 'Identification of normal and pathological aging in prospectively studied nondemented elderly humans', *Neurobiology of aging*. Elsevier, 13(1), pp. 179–189.

Ding, H., Dolan, P. J. and Johnson, G. V. W. (2008) 'Histone deacetylase 6 interacts with the microtubule-associated protein tau', *Journal of neurochemistry*. Wiley Online Library, 106(5), pp. 2119–2130.

Doty, R. L. (2003) 'Odor perception in neurodegenerative diseases', in *Handbook of olfaction and gustation*. CRC Press, pp. 850–890.

Drewes, G. *et al.* (1995) 'Microtubule-associated protein/microtubule affinity-regulating kinase (p110mark) a novel protein kinase that regulates tau-microtubule interactions and dynamic instability by phosphorylation at the Alzheimer-specific site serine 262', *Journal of Biological Chemistry*. ASBMB, 270(13), pp. 7679–7688.

Dugger, B. N. *et al.* (2016) 'The presence of select tau species in human peripheral tissues and their relation to Alzheimer's disease', *Journal of Alzheimer's Disease*. IOS Press, 51(2), pp. 345–356.

Elie, A. *et al.* (2015) 'Tau co-organizes dynamic microtubule and actin networks', *Scientific Reports*. Nature Publishing Group, 5, pp. 1–10. doi: 10.1038/srep09964.

Ellison, D. *et al.* (2012) *Neuropathology E-Book: A Reference Text of CNS Pathology*. Elsevier Health Sciences. Available at: https://books.google.ch/books?id=6zjFir2mfigC.

Falcon, B. *et al.* (2018) 'Structures of filaments from Pick's disease reveal a novel tau protein fold', *Nature*. Springer US, 561(7721), pp. 137–140. doi: 10.1038/s41586-018-0454-y.

Falcon, B. *et al.* (2019) 'HHS Public Access', *Nature*, 568(7752), pp. 420–423. doi: 10.1038/s41586-019-1026-5.Novel.

Fitzpatrick, A. W. P. *et al.* (2017) 'Cryo-EM structures of tau filaments from Alzheimer's disease.', *Nature*. Nature Publishing Group, 547(7662), pp. 185–190. doi: 10.1038/nature23002.

Frank, S., Clavaguera, F. and Tolnay, M. (2008) 'Tauopathy models and human neuropathology: similarities and differences', *Acta neuropathologica*. Springer, 115(1), pp. 39–53.

Franklin, K. and Paxinos, G. (2007) *The Mouse Brain in Stereotaxic Coordinates Third Edition*. third, *Psychology*. third. Academic Press Elsevier.

Franzmann, T. M. *et al.* (2018) 'Phase separation of a yeast prion protein promotes cellular fitness', *Science*, 359(6371). doi: 10.1126/science.aao5654.

Froger, A. and Hall, J. E. (2007) 'Transformation of plasmid DNA into E. coli using the heat shock method', *JoVE (Journal of Visualized Experiments)*, (6), p. e253.

Funk, K. E. *et al.* (2014) 'Lysine methylation is an endogenous post-translational modification of tau protein in human brain and a modulator of aggregation propensity', 88, pp. 77–88. doi: 10.1042/BJ20140372.

Gaboriau-Routhiau, V. *et al.* (2009) 'The Key Role of Segmented Filamentous Bacteria in the Coordinated Maturation of Gut Helper T Cell Responses', *Immunity*, 31(4), pp. 677–689. doi: 10.1016/j.immuni.2009.08.020.

Gallyas, F. (1971) 'Silver staining of Alzheimer's neurofibrillary changes by means of physical development', *Acta Morphol Acad Sci Hung*, 19, pp. 1–8.

Gaugler, J. *et al.* (2016) '2016 Alzheimer's disease facts and figures', *Alzheimer's and Dementia*. Elsevier Inc., 12(4), pp. 459–509. doi: 10.1016/j.jalz.2016.03.001.

Georgieva, E. R. *et al.* (2014) 'Tau binds to lipid membrane surfaces via short amphipathic helices located in its microtubule-binding repeats', *Biophysical Journal*. Biophysical Society, 107(6), pp. 1441–1452. doi: 10.1016/j.bpj.2014.07.046.

Ghetti, B. *et al.* (2015) 'Invited review: Frontotemporal dementia caused by microtubule-associated protein tau gene (MAPT) mutations: A chameleon for neuropathology and neuroimaging', *Neuropathology and Applied Neurobiology*, 41(1), pp. 24–46. doi: 10.1111/nan.12213.

Ghetti, B., Wszolek, Z. K. and Boeve, B. F. (2011) '14 Frontotemporal Dementia and Parkinsonism Linked to Chromosome 17', *Neurodegeneration: The molecular pathology of dementia and movement disorders*. John Wiley & Sons, p. 110.

Gilbert, J. A. *et al.* (2016) 'Microbiome-wide association studies link dynamic microbial consortia to disease', *Nature*. Nature Publishing Group, 535(7610), p. 94.

Girard, E. *et al.* (2015) 'Efficacy of cabazitaxel in mouse models of pediatric brain tumors', *Neuro-Oncology*, 17(1), pp. 107–115. doi: 10.1093/neuonc/nou163.

Goedert, M. *et al.* (1989) 'Multiple isoforms of human microtubule-associated protein tau: sequences and localization in neurofibrillary tangles of Alzheimer's disease', *Neuron*. Elsevier, 3(4), pp. 519–526.

Goedert, M. *et al.* (1992) 'Tau proteins of Alzheimer paired helical filaments: abnormal phosphorylation of all six brain isoforms', *Neuron*. Elsevier, 8(1), pp. 159–168.

Goedert, M. *et al.* (1996) 'PTL-1, a microtubule-associated protein with tau-like repeats from the nematode Caenorhabditis elegans', *Journal of cell science*. The Company of Biologists Ltd, 109(11), pp. 2661–2672.

Goedert, M. (2015) 'Alzheimer's and Parkinson's diseases: The prion concept in relation to assembled Aβ, tau, and α-synuclein', *Science*, 349(6248), p. 9203. doi: 10.1126/science.1255555.

Goedert, M., Eisenberg, D. S. and Crowther, R. A. (2017) 'Propagation of tau aggregates and neurodegeneration', *Annual Review of Neuroscience*. Annual Reviews, 40, pp. 189–210.

Gong, C.-X. *et al.* (2002) 'Phosphorylation of Microtubule-associated Protein Tau Is Regulated by Protein Phosphatase 2A in Mammalian Brain', *Journal of Biological Chemistry*, 275(8), pp. 5535–5544. doi: 10.1074/jbc.275.8.5535.

Gorkovskiy, A. *et al.* (2014) 'Locating folds of the in-register parallel -sheet of the Sup35p prion domain infectious amyloid', *Proceedings of the National Academy of Sciences*, 111(43), pp. E4615–E4622. doi: 10.1073/pnas.1417974111.

Guo, T., Noble, W. and Hanger, D. P. (2017) 'Roles of tau protein in health and disease', *Acta Neuropathologica*. Springer Berlin Heidelberg, 133(5), pp. 665–704. doi: 10.1007/s00401-017-1707-9.

Gupta, S. *et al.* (2010) 'Alzheimer ' s Disease : Genes , Proteins , and Therapy', 81(2), pp. 741–766.

Halfmann, R. *et al.* (2012) 'Prions are a common mechanism for phenotypic inheritance in wild yeasts', *Nature*. Nature Publishing Group, 482(7385), pp. 363–368. doi: 10.1038/nature10875.

Halpain, S. and Dehmelt, L. (2006) 'The MAP1 family of microtubule-associated proteins', *Genome Biology*, 7(6), pp. 1–7. doi: 10.1186/gb-2006-7-6-224.

Hamdane, M. *et al.* (2006) 'Pin1 allows for differential Tau dephosphorylation in neuronal cells', *Molecular and Cellular Neuroscience*. Elsevier, 32(1–2), pp. 155–160.

Hanger, D. P., Anderton, B. H. and Noble, W. (2009) 'Tau phosphorylation: the therapeutic challenge for neurodegenerative disease', *Trends in Molecular Medicine*, 15(3), pp. 112–119. doi: 10.1016/j.molmed.2009.01.003.

Hanger, D. P. and Wray, S. (2010) 'Tau cleavage and tau aggregation in neurodegenerative disease', *Biochemical Society Transactions*, 38(4), pp. 1016–1020. doi: 10.1042/BST0381016.

Hawkes, C. H., Tredici, D. and Braak, H. (2009) 'Parkinson ' s Disease The Dual Hit Theory Revisited', 622, pp. 615–622. doi: 10.1111/j.1749-6632.2009.04365.x.

He, H. J. *et al.* (2009) 'The proline-rich domain of tau plays a role in interactions with actin', *BMC Cell Biology*, 10. doi: 10.1186/1471-2121-10-81.

Hernández, F. *et al.* (2019) 'Differences in structure and function between human and murine tau', *Biochimica et Biophysica Acta - Molecular Basis of Disease*. Elsevier, 1865(8), pp. 2024–2030. doi: 10.1016/j.bbadis.2018.08.010.

Hernandez, F. and Avila, J. (2007) 'Tauopathies', *Cellular and Molecular Life Sciences*. Springer, 64(17), pp. 2219–2233.

Hernández, F., Merchán-rubira, J. and Vallés-saiz, L. (2020) 'Differences Between Human and Murine Tau at the N-terminal End', 12(January), pp. 1–6. doi: 10.3389/fnagi.2020.00011.

Himmler, A. (1989) 'Structure of the bovine tau gene: alternatively spliced transcripts generate a protein family.', *Molecular and Cellular Biology*. Am Soc Microbiol, 9(4), pp. 1389–1396.

Hogg, M. *et al.* (2003) 'The L266V tau mutation is associated with frontotemporal dementia and Pick-like 3R and 4R tauopathy', *Acta neuropathologica*. Springer, 106(4), pp. 323–336.

Hope, J. *et al.* (1988) 'Fibrils from brains of cows with new cattle disease contain scrapie-associated protein', *Nature*. Nature Publishing Group, 336(6197), p. 390.

Horvath, I. and Wittung-Stafshede, P. (2016) 'Cross-talk between amyloidogenic proteins in type-2 diabetes and Parkinson's disease', *Proceedings of the National Academy of Sciences*. National Acad Sciences, 113(44), pp. 12473–12477.

Huang, D. Y. *et al.* (1995) 'ApoE3 binding to tau tandem repeat I is abolished by tau serine262 phosphorylation', *Neuroscience letters*. Elsevier, 192(3), pp. 209–212.

Huttenhower, C. *et al.* (2012) 'Structure, function and diversity of the healthy human microbiome', *Nature*. Nature Publishing Group, 486(7402), pp. 207–214. doi: 10.1038/nature11234.

Hutton, M. *et al.* (1998) 'Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17', *Nature*. Nature Publishing Group, 393(6686), pp. 702–705.

Iis, L. (1996) 'Neurosciehc[lett[iis', *Neuroscience Letters*, 220, pp. 9–12.

Irwin, D. J. *et al.* (2013) 'Acetylated tau neuropathology in sporadic and hereditary tauopathies', *American Journal of Pathology*. American Society for Investigative Pathology, 183(2), pp. 344–351. doi: 10.1016/j.ajpath.2013.04.025.

Ittner, L. M. *et al.* (2010) 'Dendritic function of tau mediates amyloid-β toxicity in alzheimer's disease mouse models', *Cell*, 142(3), pp. 387–397. doi: 10.1016/j.cell.2010.06.036.

Ivanov, I. I. *et al.* (2009) 'Induction of Intestinal Th17 Cells by Segmented Filamentous Bacteria', *Cell*. Elsevier Ltd, 139(3), pp. 485–498. doi: 10.1016/j.cell.2009.09.033.

Jaunmuktane, Z. *et al.* (2015) 'Evidence for human transmission of amyloid-β pathology and cerebral amyloid angiopathy', *Nature*, 525(7568), pp. 247–250. doi: 10.1038/nature15369.

Jensen, P. H. *et al.* (1999) 'α-Synuclein binds to tau and stimulates the protein kinase A-catalyzed tau phosphorylation of serine residues 262 and 356', *Journal of Biological Chemistry*. ASBMB, 274(36), pp. 25481–25489.

Johan, K. *et al.* (1998) 'Acceleration of amyloid protein A amyloidosis by amyloid-like synthetic fibrils', *Proceedings of the National Academy of Sciences of the United States of America*, 95(5), pp. 2558–2563. doi: 10.1073/pnas.95.5.2558.

La Joie, R. *et al.* (2020) 'Prospective longitudinal atrophy in Alzheimer's disease correlates with the intensity and topography of baseline tau-PET.', *Science translational medicine*, 12(524), pp. 1–13. doi: 10.1126/scitranslmed.aau5732.

Kadavath, H. *et al.* (2015) 'Tau stabilizes microtubules by binding at the interface between tubulin heterodimers', *Proceedings of the National Academy of Sciences*, 112(24), pp. 7501–7506. doi: 10.1073/pnas.1504081112.

Kalaria, R. N., Akinyemi, R. and Ihara, M. (2012) 'Journal of the Neurological Sciences Does vascular pathology contribute to Alzheimer changes ?', *Journal of the Neurological Sciences*. Elsevier B.V., 322(1–2), pp. 141–147. doi: 10.1016/j.jns.2012.07.032.

Kampers, T. *et al.* (1999) 'Assembly of paired helical filaments from mouse tau: implications for the neurofibrillary pathology in transgenic mouse models for Alzheimer's disease', *FEBS letters*. Wiley Online Library, 451(1), pp. 39–44.

Kanaan, N. M. *et al.* (2011) 'Pathogenic forms of tau inhibit kinesin-dependent axonal transport through a mechanism involving activation of axonal phosphotransferases', *Journal of Neuroscience*. Soc Neuroscience, 31(27), pp. 9858–9868.

van der Kant, R., Goldstein, L. S. B. and Ossenkoppele, R. (2020) 'Amyloid-β-independent regulators of tau pathology in Alzheimer disease', *Nature Reviews Neuroscience*. Springer US, 21(1), pp. 21–35. doi: 10.1038/s41583-019-0240-3.

Karsten, S. L. *et al.* (2006) 'A genomic screen for modifiers of tauopathy identifies puromycin-sensitive aminopeptidase as an inhibitor of tau-induced neurodegeneration', *Neuron*. Elsevier, 51(5), pp. 549–560.

Kaufman, S. K. *et al.* (2016) 'Tau Prion Strains Dictate Patterns of Cell Pathology, Progression Rate, and Regional Vulnerability In Vivo', *Neuron*. Elsevier Inc., 92(4), pp. 796–812. doi: 10.1016/j.neuron.2016.09.055.

Kawas, C. *et al.* (2000) 'Age-specific incidence rates of Alzheimer's disease', *Neurology*, 54(11), pp. 2072 LP – 2077. doi: 10.1212/WNL.54.11.2072.

Kelly, A. C., Busby, B. and Wickner, R. B. (2014) 'Effect of domestication on the spread of the [PIN+] prion in Saccharomyces cerevisiae', *Genetics*, 197(3), pp. 1007–1024. doi: 10.1534/genetics.114.165670.

Keshavarzian, A. *et al.* (2015) 'Colonic bacterial composition in Parkinson's disease', *Movement Disorders*, 30(10), pp. 1351–1360. doi: 10.1002/mds.26307.

Kim, S. *et al.* (2019) 'Transneuronal Propagation of Pathologic α-Synuclein from the Gut to the Brain Models Parkinson's Disease', *Neuron*. Elsevier Inc., pp. 1–15. doi: 10.1016/j.neuron.2019.05.035.

King, C. Y. *et al.* (1997) 'Prion-inducing domain 2-114 of yeast Sup35 protein transforms in vitro into amyloid-like filaments', *Proceedings of the National Academy of Sciences of the United States of America*, 94(13), pp. 6618–6622. doi: 10.1073/pnas.94.13.6618.

Knip, M. and Siljander, H. (2016) 'The role of the intestinal microbiota in type 1 diabetes mellitus', *Nature Reviews Endocrinology*. Nature Publishing Group, 12(3), pp. 154–167. doi: 10.1038/nrendo.2015.218.

Knowles, T. P. J., Vendruscolo, M. and Dobson, C. M. (2014) 'The amyloid state and its association with protein misfolding diseases', *Nature Reviews Molecular Cell Biology*. Nature Publishing Group, 15(6), pp. 384–396. doi: 10.1038/nrm3810.

Kopke, E. *et al.* (1993) 'Microtubule-associated protein tau. Abnormal phosphorylation of a non-paired helical filament pool in Alzheimer disease', *Journal of Biological Chemistry*, 268(32), pp. 24374–24384.

Krammer, C. *et al.* (2008) 'The yeast Sup35NM domain propagates as a prion in mammalian cells'.

Kudo, L. C. *et al.* (2011) 'Puromycin-sensitive aminopeptidase (PSA/NPEPPS) impedes development of neuropathology in HPSA/TAU P301L double-transgenic mice', *Human Molecular Genetics*, 20(9), pp. 1820–1833. doi: 10.1093/hmg/ddr065.

Kushnirov, V. V. (2000) 'Prion properties of the Sup35 protein of yeast Pichia methanolica', *The EMBO Journal*, 19(3), pp. 324–331. doi: 10.1093/emboj/19.3.324.

Lafaille-Magnan, M.-E. *et al.* (2017) 'Odor identification as a biomarker of preclinical AD in older adults at risk', *Neurology*. AAN Enterprises, 89(4), pp. 327–335.

Lazzarini, R. *et al.* (2015) 'Physicochemical stability of cabazitaxel and docetaxel solutions', *European Journal of Hospital Pharmacy* , 22(3), pp. 150–155. doi: 10.1136/ejhpharm-2014-000558.

Ledesma, M. D., Bonay, P. and Avila, J. (1995) 'τ Protein from Alzheimer's disease patients is glycated at its tubulin-binding domain', *Journal of neurochemistry*. Wiley Online Library, 65(4), pp. 1658–1664.

Lee, G., Cowan, N. and Kirschner, M. (1988) 'The primary structure and heterogeneity of tau protein from mouse brain', *Science*. American Association for the Advancement of Science, 239(4837), pp. 285–288.

Lee, G. and Rook, S. L. (1992) 'Expression of tau protein in non-neuronal cells: microtubule binding and stabilization.', *Journal of cell science*, 102 (Pt 2, pp. 227–37. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1400630.

Lee, V M, Goedert, M. and Trojanowski, J. Q. (2001) 'Neurodegenerative tauopathies.', *Annual review of neuroscience*. United States, 24, pp. 1121–1159. doi: 10.1146/annurev.neuro.24.1.1121.

Leroy, K. *et al.* (2012) 'Lack of tau proteins rescues neuronal cell death and decreases amyloidogenic processing of APP in APP/PS1 mice', *American Journal of Pathology*. Elsevier Inc., 181(6), pp. 1928–1940. doi: 10.1016/j.ajpath.2012.08.012.

Lin, W. L., Dickson, D. W. and Sahara, N. (2011) 'Immunoelectron microscopic and biochemical studies of caspase-cleaved tau in a mouse model of tauopathy', *Journal of Neuropathology and Experimental Neurology*, 70(9), pp. 779–787. doi: 10.1097/NEN.0b013e31822ac9c9.

Lindquist, S. *et al.* (2004) 'Self-Seeded Fibers Formed by Sup35, the Protein Determinant of [PSI+], a Heritable Prion-like Factor of S. Cerevisiae', *Cell*, 89(5), pp. 811–819. doi: 10.1016/s0092-8674(00)80264-0.

Littman, D. R. and Pamer, E. G. (2011) 'Role of the commensal microbiota in normal and pathogenic host immune responses', *Cell Host and Microbe*, 10(4), pp. 311–323. doi: 10.1016/j.chom.2011.10.004.

Liu, B. *et al.* (2017) 'Vagotomy and Parkinson disease', *Neurology*, 88, pp. 1996–2002. doi: 10.1212/WNL.0000000000003961.

Liu, C. *et al.* (2016) 'Co-immunoprecipitation with Tau isoform-specific antibodies reveals distinct protein interactions and highlights a putative role for 2N Tau in disease', *Journal of Biological Chemistry*, 291(15), pp. 8173–8188. doi: 10.1074/jbc.M115.641902.

Liu, C. and Götz, J. (2013) 'Profiling murine tau with 0N, 1N and 2N isoform-specific antibodies in brain and peripheral organs reveals distinct subcellular localization, with the 1N isoform being enriched in the nucleus', *PloS one*. Public Library of Science, 8(12), p. e84849.

Liu, F. *et al.* (2002) 'Aberrant glycosylation modulates phosphorylation of tau by protein kinase A and dephosphorylation of tau by protein phosphatase 2A and 5', *Neuroscience*. Elsevier, 115(3), pp. 829–837.

Liu, F. *et al.* (2004) 'O-GlcNAcylation regulates phosphorylation of tau: A mechanism involved in Alzheimer's disease', *Proceedings of the National Academy of Sciences*, 101(29), pp. 10804–10809. doi: 10.1073/pnas.0400348101.

Liu, F., Grundke-Iqbal, I., *et al.* (2005) 'Contributions of protein phosphatases PP1, PP2A, PP2B and PP5 to the regulation of tau phosphorylation', *European Journal of Neuroscience*, 22(8), pp. 1942–1950. doi: 10.1111/j.1460-9568.2005.04391.x.

Liu, F., Iqbal, K., *et al.* (2005) 'Dephosphorylation of tau by protein phosphatase 5: Impairment in Alzheimer's disease', *Journal of Biological Chemistry*, 280(3), pp. 1790–1796. doi: 10.1074/jbc.M410775200.

López, O. L. and DeKosky, S. T. (2008) 'Clinical symptoms in Alzheimer's disease', *Handbook of clinical neurology*. Elsevier, 89, pp. 207–216.

Lorente de Nó, R. (1934) 'Studies on the structure of the cerebral cortex. II. Continuation of the study of the ammonic system.', *Journal für Psychologie und Neurologie*.

Lundmark, K., Westermark, G. T. and Olse, A. (2005) 'Protein fibrils in nature can enhance amyloid protein A amyloidosis in mice : Cross-seeding as a', *Proceedings of the national academy of sciences of the United States of America*.

Luo, H. *et al.* (2014) 'SUMOylation at K340 inhibits tau degradation through deregulating its phosphorylation and ubiquitination', 111(46). doi: 10.1073/pnas.1417548111.

Maina, M. B., Al-Hilaly, Y. K. and Serpell, L. C. (2016) 'Nuclear tau and its potential role in alzheimer's disease', *Biomolecules*, 6(1), pp. 2–20. doi: 10.3390/biom6010009.

Makrantonaki, E. *et al.* (2012) 'Identification of Biomarkers of Human Skin Ageing in Both Genders. Wnt Signalling - A Label of Skin Ageing?', *PLoS ONE*, 7(11), pp. 1–10. doi: 10.1371/journal.pone.0050393.

Mandelkow, E. and Mandelkow, E.-M. (1995) 'Microtubules and microtubule-associated proteins', *Current opinion in cell biology*. Elsevier, 7(1), pp. 72–81.

Martin, L., Latypova, X. and Terro, F. (2011) 'Post-translational modifications of tau protein: implications for Alzheimer's disease', *Neurochemistry international*. Elsevier, 58(4), pp. 458–471.

McAdam, K. (1978) 'Leprosy, filariasis and malaria as causes of secondary amyloidosis in the tropics.', *Papua New Guinea Medical Journal*, 21(1), pp. 69–78.

Methé, B. A. *et al.* (2012) 'A framework for human microbiome research', *Nature*. Nature Publishing Group, 486(7402), pp. 215–221. doi: 10.1038/nature11209.

Miklossy, J. *et al.* (1999) 'Alzheimer disease: curly fibers and tangles in organs other than brain', *Journal of neuropathology and experimental neurology*. American Association of Neuropathologists, Inc., 58(8), pp. 803–814.

Miller, D. B. and O'Callaghan, J. P. (2015) 'Biomarkers of Parkinson's disease: present and future', *Metabolism*. Elsevier, 64(3), pp. S40–S46.

Min, S. *et al.* (2010) 'Article Acetylation of Tau Inhibits Its Degradation and Contributes to Tauopathy', *Neuron*. Elsevier Inc., 67(6), pp. 953–966. doi: 10.1016/j.neuron.2010.08.044.

Min, S. W. *et al.* (2015) 'Critical role of acetylation in tau-mediated neurodegeneration and cognitive deficits', *Nature Medicine*. Nature Publishing Group, 21(10), pp. 1154–1162. doi: 10.1038/nm.3951.

Mirbaha, H. *et al.* (2018) 'Inert and seed-competent tau monomers suggest structural origins of aggregation', *Elife*. eLife Sciences Publications Limited, 7, p. e36584.

Moberg, P. J. and Doty, R. L. (1997) 'Olfactory function in Huntington's disease patients and at-risk offspring', *International Journal of Neuroscience*. Taylor & Francis, 89(1–2), pp. 133–139.

Morris, M. *et al.* (2011) 'The Many Faces of Tau', *Neuron*. Elsevier Inc., 70(3), pp. 410–426. doi: 10.1016/j.neuron.2011.04.009.

Morris, M. *et al.* (2015) 'Tau post-translational modifications in wild-type and human amyloid precursor protein transgenic mice', 18(8). doi: 10.1038/nn.4067.

Mukrasch, M. D. *et al.* (2007) 'The "jaws" of the tau-microtubule interaction', *Journal of Biological Chemistry*, 282(16), pp. 12230–12239. doi: 10.1074/jbc.M607159200.

Murrell, J. R. *et al.* (1999) 'Tau gene mutation G389R causes a tauopathy with abundant pick body-like inclusions and axonal deposits', *Journal of neuropathology and experimental neurology*. American Association of Neuropathologists, Inc., 58(12), pp. 1207–1226.

Musiek, E. S. and Holtzman, D. M. (2015) 'Three dimensions of the amyloid hypothesis: Time, space and "wingmen"', *Nature Neuroscience*, 18(6), pp. 800–806. doi: 10.1038/nn.4018.

Nagpal, R., Yadav, H. and Marotta, F. (2014) 'Gut Microbiota: The Next-Gen Frontier in Preventive and Therapeutic Medicine?', *Frontiers in Medicine*, 1(June), pp. 11–14. doi: 10.3389/fmed.2014.00015.

Nakagami, H. (Ed.). (2019) *Therapeutic Vaccines as Novel Immunotherapy: Biological and Clinical Concepts*. Springer Nature.

Nan, H. *et al.* (2019) 'A viral expression factor behaves as a prion', *Nature Communications*. Springer US, 10(1), pp. 1–11. doi: 10.1038/s41467-018-08180-z.

Nelson, P. T. *et al.* (1996) 'Molecular evolution of τ protein: implications for Alzheimer's disease', *Journal of neurochemistry*. Wiley Online Library, 67(4), pp. 1622–1632.

Neve, R. L. *et al.* (1986) 'Identification of cDNA clones for the human microtubule-associated protein tau and chromosomal localization of the genes for tau and microtubule-associated protein 2', *Molecular Brain Research*. Elsevier, 1(3), pp. 271–280.

Nicoll, J. A. R. *et al.* (2006) 'O RIGINAL A RTICLE AA Species Removal After AA 42 Immunization', *Experimental Neurology*, 65(11), pp. 1040–1048.

Nicoll, J. A. R. *et al.* (2019) 'Persistent neuropathological effects 14 years following amyloid-β immunization in Alzheimer's disease', *Brain*, 142(7), pp. 2113–2126. doi: 10.1093/brain/awz142.

Noel Gill, O. *et al.* (2013) 'Prevalent abnormal prion protein in human appendixes after bovine spongiform encephalopathy epizootic: Large scale survey', *BMJ (Online)*, 347(7929), pp. 1–12. doi: 10.1136/bmj.f5675.

Ohhashi, Y. *et al.* (2010) 'Differences in prion strain conformations result from non-native interactions in a nucleus', *Nature Chemical Biology*. Nature Publishing Group, 6(3), pp. 225–230. doi: 10.1038/nchembio.306.

Ohhashi, Y. *et al.* (2018) 'Molecular basis for diversification of yeast prion strain conformation', *Proceedings of the National Academy of Sciences*. National Acad Sciences, 115(10), pp. 2389–2394.

Ozben, T. and Ozben, S. (2019) 'Neuro-inflammation and anti-inflammatory treatment options for Alzheimer's disease', *Clinical Biochemistry*, 72(February), pp. 87–89. doi: 10.1016/j.clinbiochem.2019.04.001.

Ozcelik, S. *et al.* (2016) 'Co-expression of truncated and full-length tau induces severe neurotoxicity', *Molecular Psychiatry*. Nature Publishing Group, 21(12), pp. 1790–1798. doi: 10.1038/mp.2015.228.

Pagano, G. *et al.* (2018) 'Diabetes mellitus and Parkinson disease', *Neurology*. AAN Enterprises, 90(19), pp. e1654–e1662.

Paholikova, K. *et al.* (2015) 'N-terminal truncation of microtubule associated protein tau dysregulates its cellular localization', *Journal of Alzheimer's Disease*. IOS press, 43(3), pp. 915–926.

Pallarès, I., Iglesias, V. and Ventura, S. (2016) 'The rho termination factor of Clostridium botulinum contains a prion-like domain with a highly amyloidogenic core', *Frontiers in Microbiology*, 6(JAN), pp. 1–12. doi: 10.3389/fmicb.2015.01516.

Palm, N. W., de Zoete, M. R. and Flavell, R. A. (2015) 'Immune-microbiota interactions in health and disease', *Clinical Immunology*. Elsevier Inc., 159(2), pp. 122–127. doi: 10.1016/j.clim.2015.05.014.

Paushkin, S. V *et al.* (1997) 'Interaction between Yeast Sup45p (eRF1) and Sup35p (eRF3) Polypeptide Chain Release Factors : Implications for Prion-Dependent Regulation', 17(5), pp. 2798–2805.

Petrucelli, L. *et al.* (2004) 'CHIP and Hsp70 regulate tau ubiquitination , degradation and aggregation', *Human Molecular Genetics*, 13(7), pp. 703–714. doi: 10.1093/hmg/ddh083.

Pieri, L. *et al.* (2006) 'The yeast prion Ure2p native-like assemblies are toxic to mammalian cells regardless of their aggregation state', *Journal of Biological Chemistry*, 281(22), pp. 15337–15344. doi: 10.1074/jbc.M511647200.

Pisa, D. *et al.* (2015) 'Different Brain Regions are Infected with Fungi in Alzheimer's Disease', *Scientific Reports*. Nature Publishing Group, 5, pp. 1–13. doi: 10.1038/srep15015.

Poorkaj, P. *et al.* (1998) 'Tau is a candidate gene for chromosome 17 frontotemporal dementia', *Annals of neurology*. Wiley Online Library, 43(6), pp. 815–825.

Price, J. L. *et al.* (1991) 'The distribution of tangles, plaques and related immunohistochemical markers in healthy aging and Alzheimer's disease', *Neurobiology of Aging*, 12(4), pp. 295–312. doi: 10.1016/0197-4580(91)90006-6.

Probst, A. *et al.* (2000) 'Axonopathy and amyotrophy in mice transgenic for human four-repeat tau protein', *Acta neuropathologica*. Springer, 99(5), pp. 469–481.

Proctor, D. M. and Relman, D. A. (2017) 'The Landscape Ecology and Microbiota of the Human Nose, Mouth, and Throat', *Cell Host and Microbe*. Elsevier Inc., 21(4), pp. 421–432. doi: 10.1016/j.chom.2017.03.011.

Prusiner, S. B. (1982) 'Novel proteinaceous infectious particles cause scrapie', *Science*. American Association for the Advancement of Science, 216(4542), pp. 136–144.

Prusiner, S. B. (1991) 'Molecular biology of prion diseases', *Science*. American Association for the Advancement of Science, 252(5012), pp. 1515–1522.

Pryor, D. E. *et al.* (2002) 'The microtubule stabilizing agent laulimalide does not bind in the taxoid site, kills cells resistant to paclitaxel and epothilones, and may not require its epoxide moiety for activity', *Biochemistry*, 41(29), pp. 9109–9115. doi: 10.1021/bi020211b.

Purro, S. A. *et al.* (2018) 'Transmission of amyloid-β protein pathology from cadaveric pituitary growth hormone', *Nature*. Springer US, 564(7736), pp. 415–419. doi: 10.1038/s41586-018-0790-y.

Qi, H. *et al.* (2015) 'Nuclear magnetic resonance spectroscopy characterization of interaction of Tau with DNA and its regulation by phosphorylation', *Biochemistry*, 54(7), pp. 1525–1533. doi: 10.1021/bi5014613.

Qin, J. *et al.* (2010) 'A human gut microbial gene catalogue established by metagenomic sequencing', *Nature*, 464(7285), pp. 59–65. doi: 10.1038/nature08821.

Quinn, J. P. *et al.* (2018) 'Tau Proteolysis in the Pathogenesis of Tauopathies: Neurotoxic Fragments and Novel Biomarkers', *Journal of Alzheimer's disease : JAD*, 63(1), pp. 13–33. doi: 10.3233/JAD-170959.

Rademakers, R., Cruts, M. and Van Broeckhoven, C. (2004) 'The role of tau (MAPT) in frontotemporal dementia and related tauopathies', *Human Mutation*, 24(4), pp. 277–295. doi: 10.1002/humu.20086.

Rey, N. L. *et al.* (2016) 'Widespread transneuronal propagation of α-synucleinopathy triggered in olfactory bulb mimics prodromal Parkinson's disease', *Journal of Experimental Medicine*, 213(9), pp. 1759–1778. doi: 10.1084/jem.20160368.

Rey, N. L., Wesson, D. W. and Brundin, P. (2018) 'The olfactory bulb as the entry site for prion-like propagation in neurodegenerative diseases', *Neurobiology of Disease*. Van Andel Research Institute, 109, pp. 226–248. doi: 10.1016/j.nbd.2016.12.013.

Ricciarelli, R. and Fedele, E. (2017) 'The Amyloid Cascade Hypothesis in Alzheimer's Disease: It's Time to Change Our Mind', *Current Neuropharmacology*, 15(6), pp. 926–935. doi: 10.2174/1570159x15666170116143743.

Risacher, S. L. *et al.* (2017) 'Olfactory identification in subjective cognitive decline and mild cognitive impairment: Association with tau but not amyloid positron emission tomography', *Alzheimer's and Dementia: Diagnosis, Assessment and Disease Monitoring*, 9, pp. 57–66. doi: 10.1016/j.dadm.2017.09.001.

Ross, G. W. *et al.* (2006) 'Association of olfactory dysfunction with incidental Lewy bodies', *Movement Disorders*, 21(12), pp. 2062–2067. doi: 10.1002/mds.21076.

Sahara, N., Maeda, S. and Takashima, A. (2008) 'Tau oligomerization: a role for tau aggregation intermediates linked to neurodegeneration', *Current Alzheimer Research*. Bentham Science Publishers, 5(6), pp. 591–598.

Sampson, T. R. *et al.* (2020) 'A gut bacterial amyloid promotes α-synuclein aggregation and motor impairment in mice.', *eLife*, 9, pp. 1–19. doi: 10.7554/eLife.53111.

Sanders, D. W. *et al.* (2014) 'Distinct tau prion strains propagate in cells and mice and define different tauopathies', *Neuron*. Elsevier Inc., 82(6), pp. 1271–1288. doi: 10.1016/j.neuron.2014.04.047.

Santiago, J. A. and Potashkin, J. A. (2014) 'Neurobiology of Disease System-based approaches to decode the molecular links in Parkinson ' s disease and diabetes', *Neurobiology of Disease*. Elsevier Inc., 72, pp. 84–91. doi: 10.1016/j.nbd.2014.03.019.

Saunders, A. M. *et al.* (1993) 'Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease.', *Neurology*, 43(8), pp. 1467–72. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8350998.

Sawaya, M. R. *et al.* (2007) 'Atomic structures of amyloid cross-β spines reveal varied steric zippers', *Nature*. Nature Publishing Group, 447(7143), pp. 453–457.

Scheckel, C. and Aguzzi, A. (2018) 'Prions, prionoids and protein misfolding disorders', *Nature Reviews Genetics*. Springer US, 19(7), pp. 405–418. doi: 10.1038/s41576-018-0011-4.

Schönheit, B., Zarski, R. and Ohm, T. G. (2004) 'Spatial and temporal relationships between plaques and tangles in Alzheimer-pathology', *Neurobiology of Aging*, 25(6), pp. 697–711. doi: 10.1016/j.neurobiolaging.2003.09.009.

Shafquat, A. *et al.* (2014) 'Functional and phylogenetic assembly of microbial communities in the human microbiome', *Trends in Microbiology*. Elsevier Ltd, 22(5), pp. 261–266. doi: 10.1016/j.tim.2014.01.011.

Shin, R. W. *et al.* (1991) 'Hydrated autoclave pretreatment enhances tau immunoreactivity in formalin-fixed normal and Alzheimer's disease brain tissues.', *Laboratory investigation; a journal of technical methods and pathology*, 64(5), pp. 693–702.

Sillen, A. *et al.* (2007) 'NMR investigation of the interaction between the neuronal protein Tau and the microtubules', *Biochemistry*, 46(11), pp. 3055–3064. doi: 10.1021/bi061920i.

Smith, D. R. *et al.* (2017) 'The production of curli amyloid fibers is deeply integrated into the biology of escherichia coli', *Biomolecules*, 7(4). doi: 10.3390/biom7040075.

Solomon, A. *et al.* (2007) 'Amyloidogenic potential of foie gras', *Proceedings of the National Academy of Sciences of the United States of America*, 104(26), pp. 10998–11001. doi: 10.1073/pnas.0700848104.

Sparrer, H. E. *et al.* (2000) 'Evidence for the prion hypothesis: Induction of the yeast [PSI +] factor by in vitro-converted Sup35 protein', *Science*, 289(5479), pp. 595–599. doi: 10.1126/science.289.5479.595.

Stefansson, H. *et al.* (2005) 'A common inversion under selection in Europeans', *Nature Genetics*, 37(2), pp. 129–137. doi: 10.1038/ng1508.

Sturchler-Pierrat, C. *et al.* (1997) 'Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology', *Proceedings of the National Academy of Sciences of the United States of America*, 94(24), pp. 13287–13292. doi: 10.1073/pnas.94.24.13287.

Tacik, P. *et al.* (2015) 'A novel tau mutation, p. K317N, causes globular glial tauopathy', *Acta neuropathologica*. Springer, 130(2), pp. 199–214.

Takashima, A. *et al.* (1998) 'Presenilin 1 associates with glycogen synthase kinase-3β and its substrate tau', *Proceedings of the national academy of sciences*. National Acad Sciences, 95(16), pp. 9637–9641.

Takeda, A. *et al.* (2014) 'Olfactory dysfunction and dementia in Parkinson's disease', *Journal of Parkinson's disease*. IOS Press, 4(2), pp. 181–187.

Thurston, V. C., Zinkowski, R. P. and Binder, L. I. (1996) 'Tau as a nucleolar protein in human nonneural cells in vitro and in vivo', *Chromosoma*, 105(1), pp. 20–30. doi: 10.1007/BF02510035.

Tonomura, S. *et al.* (2016) 'Intracerebral hemorrhage and deep microbleeds associated with cnm-positive Streptococcus mutans; A hospital cohort study', *Scientific Reports*. Nature Publishing Group, 6(February), pp. 1–8. doi: 10.1038/srep20074.

Tsai, R. M. *et al.* (2019) 'Reactions to Multiple Ascending Doses of the Microtubule Stabilizer TPI-287 in Patients with Alzheimer Disease, Progressive Supranuclear Palsy, and Corticobasal Syndrome: A Randomized Clinical Trial', *JAMA Neurology*, 94158, pp. 1–10. doi: 10.1001/jamaneurol.2019.3812.

Tuite, M. F. (2000) 'Yeast prions and their prion-forming domain', *Cell*. Elsevier, 100(3), pp. 289–292.

Turnbaugh, P. J. *et al.* (2007) 'The Human Microbiome Project', *Nature*, 449(7164), pp. 804–810. doi: 10.1038/nature06244.

Tysnes, O. B. *et al.* (2015) 'Does vagotomy reduce the risk of Parkinson's disease?', *Annals of Neurology*, 78(6), pp. 1011–1012. doi: 10.1002/ana.24531.

Uesaka, T. *et al.* (2016) 'Development of the intrinsic and extrinsic innervation of the gut', *Developmental Biology*. Elsevier, 417(2), pp. 158–167. doi: 10.1016/j.ydbio.2016.04.016.

Ulrich, J. (1985) 'Alzheimer changes in nondemented patients younger than sixty-five: Possible early stages of Alzheimer's disease and senile dementia of Alzheimer type', *Annals of neurology*. Wiley Online Library, 17(3), pp. 273–277.

Valbuena, I. M., Villegas, I. A. and Azcarate, R. V. (2018) 'Interaction of amyloidogenic proteins in pancreatic β cells from subjects with synucleinopathies', *Acta Neuropathologica*. Springer Berlin Heidelberg, 135(6), pp. 877–886. doi: 10.1007/s00401-018-1832-0.

Villemagne, V. L. *et al.* (2011) 'Longitudinal assessment of Aβ and cognition in aging and Alzheimer disease', *Annals of Neurology*, 69(1), pp. 181–192. doi: 10.1002/ana.22248.

Walker, L. C. *et al.* (2013) 'Mechanisms of protein seeding in neurodegenerative diseases', *JAMA neurology*. American Medical Association, 70(3), pp. 304–310.

Wang, A. C. *et al.* (2016) ' Loss of O -GlcNAc glycosylation in forebrain excitatory neurons induces neurodegeneration ', *Proceedings of the National Academy of Sciences*, 113(52), pp. 15120–15125. doi: 10.1073/pnas.1606899113.

Wang, J.-Z., Grundke-Iqbal, I. and Iqbal, K. (1996) 'Glycosylation of microtubule–associated protein tau: An abnormal posttranslational modification in Alzheimer's disease', *Nature medicine*. Nature Publishing Group, 2(8), p. 871.

Wang, X. S. *et al.* (2006) 'The proline-rich domain and the microtubule binding domain of protein tau acting as RNA binding domains', *Protein and peptide letters*. Bentham Science Publishers, 13(7), pp. 679–685.

Weingarten, M. D. *et al.* (1975) 'A protein factor essential for microtubule assembly', *Proceedings of the National Academy of Sciences*. National Acad Sciences, 72(5), pp. 1858–1862.

Westergard, L. and True, H. L. (2014) 'Wild yeast harbour a variety of distinct amyloid structures with strong prion-inducing capabilities', *Molecular Microbiology*, 92(March), pp. 183–193. doi: 10.1111/mmi.12543.

Wickner, R. B. (1994) '[URE3] as an altered URE2 protein: Evidence for a prion analog in Saccharomyces cerevisiae', *Science*, 264(5158), pp. 566–569. doi: 10.1126/science.7909170.

Williams, D. R. (2006) 'Tauopathies: Classification and clinical update on neurodegenerative diseases associated with microtubule-associated protein tau', *Internal Medicine Journal*, 36(10), pp. 652–660. doi: 10.1111/j.1445-5994.2006.01153.x.

Williams, D. R. and Lees, A. J. (2009) 'Progressive supranuclear palsy: clinicopathological concepts and diagnostic challenges', *The Lancet Neurology*. Elsevier Ltd, 8(3), pp. 270–279. doi: 10.1016/S1474-4422(09)70042-0.

Wyss-Coray, T. and Rogers, J. (2012) 'Inflammation in Alzheimer disease-A brief review of the basic science and clinical literature', *Cold Spring Harbor Perspectives in Medicine*, 2(1), pp. 1–24. doi: 10.1101/cshperspect.a006346.

Xu, Q. *et al.* (2011) 'Diabetes and risk of Parkinson's disease', *Diabetes Care*, 34(4), pp. 910–915. doi: 10.2337/dc10-1922.

Yang, X. and Seto, E. (2008) 'Review Lysine Acetylation : Codified Crosstalk with Other Posttranslational Modifications', 11(class IV), pp. 449–461. doi: 10.1016/j.molcel.2008.07.002.

Yoon, J. H. *et al.* (2015) 'Olfactory function and neuropsychological profile to differentiate dementia with Lewy bodies from Alzheimer's disease in patients with mild cognitive impairment: A 5-year follow-up study', *Journal of the Neurological Sciences*. Elsevier B.V., 355(1–2), pp. 174–179. doi: 10.1016/j.jns.2015.06.013.

Yuan, A. H. and Hochschild, A. (2017) 'A bacterial global regulator forms a prion', *Science*, 355(6321), p. 9203. doi: 10.1126/science.aai7776.

Yuan, J. *et al.* (2006) 'Insoluble aggregates and protease-resistant conformers of prion protein in uninfected human brains', *Journal of Biological Chemistry*, 281(46), pp. 34848–34858. doi: 10.1074/jbc.M602238200.

Yuzwa, S. A. *et al.* (2014) 'O-GlcNAc Modification of tau Directly Inhibits Its Aggregation without Perturbing the Conformational Properties of tau Monomers', *Journal of Molecular Biology*. Elsevier Ltd, 426(8), pp. 1736–1752. doi: 10.1016/j.jmb.2014.01.004.

Zhang, B. *et al.* (2012) 'The microtubule-stabilizing agent, epothilone D, reduces axonal dysfunction, neurotoxicity, cognitive deficits, and Alzheimer-like pathology in an interventional study with aged tau transgenic mice', *Journal of Neuroscience*. Soc Neuroscience, 32(11), pp. 3601–3611.

Zhang, W. *et al.* (2019) 'Novel tau filament fold in corticobasal degeneration, a four-repeat tauopathy', *bioRxiv*. Springer US, p. 811703. doi: 10.1101/811703.

Zhang, Y. and Zhang, H. (2013) 'Microbiota associated with type 2 diabetes and its related complications', *Food Science and Human Wellness*. Beijing Academy of Food Sciences., 2(3–4), pp. 167–172. doi: 10.1016/j.fshw.2013.09.002.

Zhang, Z. *et al.* (2014) 'Cleavage of tau by asparagine endopeptidase mediates the neurofibrillary pathology in Alzheimer's disease', *Nature medicine*, 20(11), pp. 1254–1262. doi: 10.1038/nm.3700.